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(57) Abstract

The present invention relates to polynucleotide and polypeptide molecules for zsig28, a novel member of the RPV.1 family of proteins. The polynucleotides encoding zsig28 can be used to identify a region of the genome associated with human disease states. The present invention also includes methods for producing the protein, uses therefor and antibodies thereto.

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Description

STOMACH POLYPEPTIDE ZSIG28

BACKGROUND OF THE INVENTION

Proper control of the opposing processes of cell proliferation versus terminal differentiation and apoptotic programmed cell death is an important aspect of normal development and homeostasis (Raff, M.C., Cell 86:173-175, 1996), and has been found to be altered in many human diseases. See, for example, Sawyers, C.L. et al., Cell 64:337-350, 1991; Meyaard, L. et al., Science 257:217-219, 1992; Guo, Q. et al., Nature Med. 4:957-962, 1998; Barinaga, M., Science, 273:735-737, 1996; Solary, E. et al., Eur. Respir. J., 9:1293-1305, 1996; Hamet, P. et al., J. Hypertension, 14:S65-S70, 1996; Roy, N. et al., Cell, 80:167-178, 1995; and Ambrosini, G., Nature Med., 8:917-921, 1997. Much progress has been made towards understanding the regulation of this balance. For example, signaling cascades have been elucidated through which extracellular stimuli, such as growth factors, peptide hormones, and cell-cell interactions, control the commitment of precursor cells to specific cell lineages and their subsequent proliferative expansion (Morrison, S.J. et al., Cell 88:287-298, 1997). Further, it has been found that cell cycle exit and terminal differentiation are coupled in most cell types. See, for example, Coppola, J.A. et al., Nature 320:760-763, 1986; Freytag, S.O, Mol. Cell. Biol. 8:1614-1624, 1988; Lee, E.Y. et al., Genes Dev. 8:2008-2021, 1994; Morgenbesser, S.D. et al., Nature 371:72-74, 1994; Casaccia-Bonnefil, P. et al., Genes Dev. 11:2335-2346, 1996; Zacksenhaus, E. et al., Genes Dev. 10:3051-3064, 1996; and Zhang, P. et al.,

Nature 387:151-158, 1997. Apoptosis also plays an important role in many developmental and homeostatic processes (Raff, M.C., Nature 356:397-400, 1992; Raff, M.C., supra.), and is often coordinately regulated with terminal differentiation (Jacobsen, K.A. et al., Blood 84:2784-2794, 1994; Morgenbesser et al., supra.; Yan, Y. et al., Genes Dev. 11:973-983, 1997; Zacksenhaus et al., supra.). Hence, it appears that the development of individual lineages, tissues, organs, or even entire multicellular organisms is the result of a finely tuned balance between increased cell production due to proliferation, and decreased numbers of cells resulting from terminal differentiation and apoptosis. This balance is most likely regulated coordinately by the convergence of multiple regulatory pathways. The identification of novel members of such networks can provide important insights into both normal cellular processes, as well as the etiology and treatment of human disease states.

Thus, there is a continuing need to discover new proteins that regulate proliferation, differentiation, and apoptotic pathways. The *in vivo* activities of both inducers and inhibitors of these pathways illustrates the enormous clinical potential of, and need for, novel proliferation, differentiation, and apoptotic proteins, their agonists and antagonists. The present invention addresses this need by providing such polypeptides for these and other uses that should be apparent to those skilled in the art from the teachings herein.

SUMMARY OF THE INVENTION

Within one aspect, the present invention provides an isolated polynucleotide that encodes a polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group consisting of: (a) the amino acid

sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and (b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val), wherein the amino acid percent identity is determined using a FASTA program with ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62, with other parameters set as default. Within one embodiment, the isolated polynucleotide disclosed above is selected from the group consisting of: (a) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 139 to nucleotide 853; and (b) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 70 to nucleotide 853. Within another embodiment, the isolated polynucleotide disclosed above comprises nucleotide 1 to nucleotide 783 of SEQ ID NO:10. Within another embodiment, the isolated polynucleotide disclosed above comprises a sequence of amino acid residues selected from the group consisting of: (a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and (b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val).

Within a second aspect, the present invention provides an expression vector comprising the following operably linked elements: a transcription promoter; a DNA segment encoding a zsig28 polypeptide as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and a transcription terminator, wherein the promoter is operably linked to the DNA segment, and the DNA segment is operably linked to the transcription terminator. Within one embodiment, the expression vector disclosed above further comprises a secretory signal sequence operably linked to the DNA segment.

Within a third aspect, the present invention provides a cultured cell comprising an expression vector as disclosed above, wherein the cell expresses a polypeptide encoded by the DNA segment.

Within another aspect, the present invention provides a DNA construct encoding a fusion protein, the DNA construct comprising: a first DNA segment encoding a polypeptide comprising a sequence of amino acid residues selected from the group consisting of: (a) the amino acid sequence of SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 23 (Ala); (b) the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala) to amino acid number 82 (Leu); (c) the amino acid sequence of SEQ ID NO:2 from amino acid number 101 (Leu) to amino acid number 122 (Gly); (d) the amino acid sequence of SEQ ID NO:2 from amino acid number 141 (Asn) to amino acid number 174 (Ala); (e) the amino acid sequence of SEQ ID NO:2 from amino acid number 193 (Cys) to amino acid number 261 (Val); and (f) the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and at least one other DNA segment encoding an additional polypeptide, wherein the first and other DNA segments are connected in-frame; and wherein the first and other DNA segments encode the fusion protein.

Within another aspect, the present invention provides an expression vector comprising the following operably linked elements: a transcription promoter; a DNA construct encoding a fusion protein as disclosed above; and a transcription terminator, wherein the promoter is operably linked to the DNA construct, and the DNA construct is operably linked to the transcription terminator.

Within another aspect, the present invention provides a cultured cell comprising an expression vector

as disclosed above, wherein the cell expresses a polypeptide encoded by the DNA construct.

Within another aspect, the present invention provides a method of producing a fusion protein comprising: culturing a cell as disclosed above; and isolating the polypeptide produced by the cell.

Within another aspect, the present invention provides an isolated polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group consisting of: (a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and (b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val), wherein the amino acid percent identity is determined using a FASTA program with ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62, with other parameters set as default. Within one embodiment, the isolated polypeptide disclosed above further contains motifs 1 through 4 spaced apart from N-terminus to C-terminus in a configuration selected from the group consisting of: (a) Met-{47-50}-M1-{21-22}-M2-{73-92}-M3; and (b) Met-{47-50}-M1-{21-22}-M2-{73-92}-M3-{3}-M4, wherein M1 is "motif 1," a sequence of amino acids as shown in amino acids 48 to 54 of SEQ ID NO:2, M2 is "motif 2," a sequence of amino acids as shown in amino acids 77 to 82 of SEQ ID NO:2, M3 is "motif 3," a sequence of amino acids as shown in amino acids 174 to 180 of SEQ ID NO:2, M4 is "motif 4," a sequence of amino acids as shown in amino acids 184 to 189 of SEQ ID NO:2, and {#} denotes the number of amino acids between the motifs. Within another embodiment, the isolated polypeptide disclosed above comprises a sequence of amino acid residues selected from the group consisting of: (a) the amino acid sequence as shown in SEQ ID NO:2 from amino

acid number 24 (Ala), to amino acid number 261 (Val); and (b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val).

Within another aspect, the present invention provides a method of producing a zsig28 polypeptide comprising: culturing a cell as disclosed above; and isolating the zsig28 polypeptide produced by the cell.

Within another aspect, the present invention provides a method of producing an antibody to zsig28 polypeptide comprising: inoculating an animal with a polypeptide selected from the group consisting of: (a) a polypeptide consisting of 9 to 238 amino acids, wherein the polypeptide is identical to a contiguous sequence of amino acids in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); (b) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala) to amino acid number 82 (Leu); (c) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 101 (Leu) to amino acid number 122 (Gly); (d) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 141 (Asn) to amino acid number 174 (Ala); (e) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 193 (Cys) to amino acid number 261 (Val); (f) a polypeptide as disclosed above; (g) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 245 (Ala) to amino acid number 250 (Glu); (h) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 234 (Asn) to amino acid number 239 (Lys); (i) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 202 (Glu) to amino acid number 207 (Lys); (j) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number

254 (Lys) to amino acid number 259 (Asp); and (k) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 110 (Glu) to amino acid number 115 (Ala); and wherein the polypeptide elicits an immune response in the animal to produce the antibody; and isolating the antibody from the animal.

Within another aspect, the present invention provides an antibody produced by the method disclosed above, which binds to a zsig28 polypeptide. Within one embodiment, the antibody disclosed above is a monoclonal antibody.

Within another aspect, the present invention provides an antibody which specifically binds to a polypeptide disclosed above.

Within another aspect, the present invention provides a method of detecting, in a test sample, the presence of a modulator of zsig28 protein activity, comprising: culturing a cell into which has been introduced an expression vector as disclosed above, wherein the cell expresses the zsig28 protein encoded by the DNA segment in the presence and absence of a test sample; and comparing levels of activity of zsig28 in the presence and absence of a test sample, by a biological or biochemical assay; and determining from the comparison, the presence of modulator of zsig28 activity in the test sample.

These and other aspects of the invention will become evident upon reference to the following detailed description of the invention and attached drawings.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is an alignment of zsig28 (SEQ ID NO:2); murine claudin 1 (CLAUD1) (SEQ ID NO:3) (Furuse, M. et al., J. Cell Biol. 141:1539-1550, 1998); Genbank Accession No. AF072127); Murine CPE receptor (AB007) (SEQ

ID NO:4) (Genbank Accession No. AB00713); human oligodendrocyte-specific protein (OSP)-like protein (HSU899) (SEQ ID NO:5) (Genbank Accession No. U89916); human transmembrane protein deleted in Velo-Cardio-Facial Syndrome (AF0009) (SEQ ID NO:6) (Genbank Accession No. AF000959); human OSP (AF0688) (SEQ ID NO:7) (Genbank Accession No. AF068863); murine claudin 2 (AF0721) (SEQ ID NO:8) (Furuse, M. et al., supra.; Genbank Accession No. AF072128); and rat androgen withdrawal protein RVP.1 (PIR_A3) (SEQ ID NO:9) (Briehl, M.M. et al., Mol. Endocrinol. 5:1381-1388, 1991).

Figure 2 is a hydrophobicity plot of zsig28 polypeptide.

DETAILED DESCRIPTION OF THE INVENTION

Prior to setting forth the invention in detail, it may be helpful to the understanding thereof to define the following terms:

The term "affinity tag" is used herein to denote a polypeptide segment that can be attached to a second polypeptide to provide for purification or detection of the second polypeptide or provide sites for attachment of the second polypeptide to a substrate. In principal, any peptide or protein for which an antibody or other specific binding agent is available can be used as an affinity tag. Affinity tags include a poly-histidine tract, protein A (Nilsson et al., EMBO J. 4:1075, 1985; Nilsson et al., Methods Enzymol. 198:3, 1991), glutathione S transferase (Smith and Johnson, Gene 67:31, 1988), Glu-Glu affinity tag (Grussenmeyer et al., Proc. Natl. Acad. Sci. USA 82:7952-4, 1985), substance P, Flag™ peptide (Hopp et al., Biotechnology 6:1204-10, 1988), streptavidin binding peptide, or other antigenic epitope or binding domain. See, in general, Ford et al., Protein Expression and Purification 2: 95-107, 1991. DNAs encoding affinity tags

are available from commercial suppliers (e.g., Pharmacia Biotech, Piscataway, NJ).

The term "allelic variant" is used herein to denote any of two or more alternative forms of a gene occupying the same chromosomal locus. Allelic variation arises naturally through mutation, and may result in phenotypic polymorphism within populations. Gene mutations can be silent (no change in the encoded polypeptide) or may encode polypeptides having an altered amino acid sequence. The term allelic variant is also used herein to denote a protein encoded by an allelic variant of a gene.

The terms "amino-terminal" and "carboxyl-terminal" are used herein to denote positions within polypeptides. Where the context allows, these terms are used with reference to a particular sequence or portion of a polypeptide to denote proximity or relative position. For example, a certain sequence positioned carboxyl-terminal to a reference sequence within a polypeptide is located proximal to the carboxyl terminus of the reference sequence, but is not necessarily at the carboxyl terminus of the complete polypeptide.

The term "complement/anti-complement pair" denotes non-identical moieties that form a non-covalently associated, stable pair under appropriate conditions. For instance, biotin and avidin (or streptavidin) are prototypical members of a complement/anti-complement pair. Other exemplary complement/anti-complement pairs include receptor/ligand pairs, antibody/antigen (or hapten or epitope) pairs, sense/antisense polynucleotide pairs, and the like. Where subsequent dissociation of the complement/anti-complement pair is desirable, the complement/anti-complement pair preferably has a binding affinity of $<10^9\ M^{-1}$.

The term "complements of a polynucleotide molecule" denotes a polynucleotide molecule having a complementary base sequence and reverse orientation as

compared to a reference sequence. For example, the sequence 5' ATGCACGGG 3' is complementary to 5' CCCGTGCAT 3'.

The term "contig" denotes a polynucleotide that has a contiguous stretch of identical or complementary sequence to another polynucleotide. Contiguous sequences are said to "overlap" a given stretch of polynucleotide sequence either in their entirety or along a partial stretch of the polynucleotide. For example, representative contigs to the polynucleotide sequence 5'-ATGGAGCTT-3' are 5'-AGCTTgagt-3' and 3'-tcgacTACC-5'.

The term "degenerate nucleotide sequence" denotes a sequence of nucleotides that includes one or more degenerate codons (as compared to a reference polynucleotide molecule that encodes a polypeptide). Degenerate codons contain different triplets of nucleotides, but encode the same amino acid residue (i.e., GAU and GAC triplets each encode Asp).

A "DNA segment" is a portion of a larger DNA molecule having specified attributes. For example, a DNA segment encoding a specified polypeptide is a portion of a longer DNA molecule, such as a plasmid or plasmid fragment, that, when read from the 5' to the 3' direction, encodes the sequence of amino acids of the specified polypeptide.

The term "expression vector" is used to denote a DNA molecule, linear or circular, that comprises a segment encoding a polypeptide of interest operably linked to additional segments that provide for its transcription. Such additional segments include promoter and terminator sequences, and may also include one or more origins of replication, one or more selectable markers, an enhancer, a polyadenylation signal, etc. Expression vectors are generally derived from plasmid or viral DNA, or may contain elements of both.

The term "isolated", when applied to a polynucleotide, denotes that the polynucleotide has been removed from its natural genetic milieu and is thus free of other extraneous or unwanted coding sequences, and is in a form suitable for use within genetically engineered protein production systems. Such isolated molecules are those that are separated from their natural environment and include cDNA and genomic clones. Isolated DNA molecules of the present invention are free of other genes with which they are ordinarily associated, but may include naturally occurring 5' and 3' untranslated regions such as promoters and terminators. The identification of associated regions will be evident to one of ordinary skill in the art (see for example, Dynan and Tijan, Nature 316:774-78, 1985).

An "isolated" polypeptide or protein is a polypeptide or protein that is found in a condition other than its native environment, such as apart from blood and animal tissue. In a preferred form, the isolated polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin. It is preferred to provide the polypeptides in a highly purified form, i.e. greater than 95% pure, more preferably greater than 99% pure. When used in this context, the term "isolated" does not exclude the presence of the same polypeptide in alternative physical forms, such as dimers or alternatively glycosylated or derivatized forms.

The term "operably linked", when referring to DNA segments, indicates that the segments are arranged so that they function in concert for their intended purposes, e.g., transcription initiates in the promoter and proceeds through the coding segment to the terminator.

The term "ortholog" denotes a polypeptide or protein obtained from one species that is the functional counterpart of a polypeptide or protein from a different species. Sequence differences among orthologs are the result of speciation.

"Paralogs" are distinct but structurally related proteins made by an organism. Paralogs are believed to arise through gene duplication. For example, α-globin, β-globin, and myoglobin are paralogs of each other.

A "polynucleotide" is a single- or double-stranded polymer of deoxyribonucleotide or ribonucleotide bases read from the 5' to the 3' end. Polynucleotides include RNA and DNA, and may be isolated from natural sources, synthesized *in vitro*, or prepared from a combination of natural and synthetic molecules. Sizes of polynucleotides are expressed as base pairs (abbreviated "bp"), nucleotides ("nt"), or kilobases ("kb"). Where the context allows, the latter two terms may describe polynucleotides that are single-stranded or double-stranded. When the term is applied to double-stranded molecules it is used to denote overall length and will be understood to be equivalent to the term "base pairs". It will be recognized by those skilled in the art that the two strands of a double-stranded polynucleotide may differ slightly in length and that the ends thereof may be staggered as a result of enzymatic cleavage; thus all nucleotides within a double-stranded polynucleotide molecule may not be paired.

A "polypeptide" is a polymer of amino acid residues joined by peptide bonds, whether produced naturally or synthetically. Polypeptides of less than about 10 amino acid residues are commonly referred to as "peptides".

The term "promoter" is used herein for its art-recognized meaning to denote a portion of a gene containing DNA sequences that provide for the binding of RNA polymerase and initiation of transcription. Promoter sequences are commonly, but not always, found in the 5' non-coding regions of genes.

A "protein" is a macromolecule comprising one or more polypeptide chains. A protein may also comprise non-

peptidic components, such as carbohydrate groups. Carbohydrates and other non-peptidic substituents may be added to a protein by the cell in which the protein is produced, and will vary with the type of cell. Proteins are defined herein in terms of their amino acid backbone structures; substituents such as carbohydrate groups are generally not specified, but may be present nonetheless.

The term "receptor" denotes a cell-associated protein that binds to a bioactive molecule (i.e., a ligand) and mediates the effect of the ligand on the cell. Membrane-bound receptors are characterized by a multi-peptide structure comprising an extracellular ligand-binding domain and an intracellular effector domain that is typically involved in signal transduction. Binding of ligand to receptor results in a conformational change in the receptor that causes an interaction between the effector domain and other molecule(s) in the cell. This interaction in turn leads to an alteration in the metabolism of the cell. Metabolic events that are linked to receptor-ligand interactions include gene transcription, phosphorylation, dephosphorylation, increases in cyclic AMP production, mobilization of cellular calcium, mobilization of membrane lipids, cell adhesion, hydrolysis of inositol lipids and hydrolysis of phospholipids. In general, receptors can be membrane bound, cytosolic or nuclear; monomeric (e.g., thyroid stimulating hormone receptor, beta-adrenergic receptor) or multimeric (e.g., PDGF receptor, growth hormone receptor, IL-3 receptor, GM-CSF receptor, G-CSF receptor, erythropoietin receptor and IL-6 receptor).

The term "secretory signal sequence" denotes a DNA sequence that encodes a polypeptide (a "secretory peptide") that, as a component of a larger polypeptide, directs the larger polypeptide through a secretory pathway of a cell in which it is synthesized. The larger polypeptide is commonly cleaved to remove the secretory peptide during transit through the secretory pathway.

The term "splice variant" is used herein to denote alternative forms of RNA transcribed from a gene. Splice variation arises naturally through use of alternative splicing sites within a transcribed RNA molecule, or less commonly between separately transcribed RNA molecules, and may result in several mRNAs transcribed from the same gene. Splice variants may encode polypeptides having altered amino acid sequence. The term splice variant is also used herein to denote a protein encoded by a splice variant of an mRNA transcribed from a gene.

Molecular weights and lengths of polymers determined by imprecise analytical methods (e.g., gel electrophoresis) will be understood to be approximate values. When such a value is expressed as "about" X or "approximately" X, the stated value of X will be understood to be accurate to ±10%.

All references cited herein are incorporated by reference in their entirety.

The present invention is based in part upon the discovery of a novel DNA sequence that encodes a polypeptide having homology to a diverse family of receptor proteins, containing proteins such as human claudin 1 and 2 (Furuse, M. et al., J. Cell Biol. 141:1539-1550, 1998), human and murine oligodendrocyte-specific protein (OSP) (Bronstein, J.M. et al., Neurology 47:772-778, 1996), rat androgen-withdrawal apoptosis protein RVP.1 (Briehl, M.M., and Miesfeld, R.L., Mol. Endocrinol. 5:1381-1388, 1991), and others. Analysis of the tissue distribution of the mRNA corresponding to this novel DNA showed high expression in stomach, and low expression in lung. The polypeptide has been designated zsig28.

The novel zsig28 polypeptides of the present invention were initially identified by querying an EST database for proteins homologous to proteins having a

secretory signal sequence. These proteins are characterized by an upstream methionine start site and a hydrophobic region of approximately 13 amino acids, followed by a peptide signal peptidase cleavage site. An EST database was queried for novel DNA sequences whose translations would meet these search criteria. An EST was found and its corresponding cDNA was sequenced. The novel polypeptide encoded by the cDNA showed homology with rat RVP.1. Based on this homology, the zsig28 nucleotide sequence encodes the entire coding sequence of the predicted protein. Zsig28 may be a novel protein involved in an apoptotic cellular pathway, cell-cell signaling molecule, growth factor receptor, or extracellular matrix associated protein with growth factor hormone activity, or the like, and is a novel member of the claudin/OSP family of proteins.

The sequence of the zsig28 polypeptide was obtained from a single clone that contained its corresponding polynucleotide sequence. The clone was obtained from a lung library. Other libraries that might also be searched for such sequences include stomach, fetal lung, epithelial tissues, and the like.

The nucleotide sequence of a representative zsig28-encoding DNA is described in SEQ ID NO:1, and its deduced 261 amino acid sequence is described in SEQ ID NO:2. In its entirety, the zsig28 polypeptide (SEQ ID NO:2) represents a full-length polypeptide segment (residue 1 (Met) to residue 261 (Val) of SEQ ID NO:2). The domains and structural features of zsig28 are further described below.

Analysis of the zsig28 polypeptide encoded by the DNA sequence of SEQ ID NO:1 revealed an open reading frame encoding 261 amino acids (SEQ ID NO:2) comprising a predicted secretory signal peptide of 23 amino acid

residues (residue 1 (Met) to residue 23 (Ala) of SEQ ID NO:2), and a mature polypeptide of 238 amino acids (residue 24 (Ala) to residue 261 (Val) of SEQ ID NO:2). The zsig28 polypeptide contains three transmembrane domains:

(1) the first transmembrane domain is from amino acid 83 (Met) to amino acid 100 (Ala) of SEQ ID NO:2;

(2) the second transmembrane domain is from amino acid 123 (Ile) to amino acid 140 (Ala) of SEQ ID NO:2; and

(3) the third transmembrane domain is from amino acid 175 (Leu) to amino acid 192 (Met) of SEQ ID NO:2.

These transmembrane domains are corroborated by the zsig28 hydrophobicity plot (See, Figure 2). Between and flanking these transmembrane domains lies regions of the zsig28 molecule that may confer binding to a ligand, cell-cell interactions, cellular signaling functions, and the like. Moreover, such regions, and stretches of hydrophilic amino acids within, would serve as suitable antigenic epitopes for the production of antibodies, as discussed herein. These regions include:

(1) "region 1," the amino-terminal region, amino acid 24 (Ala) to amino acid 82 (Leu);

(2) "region 2," Amino acid 101 (Leu) to amino acid 122 (Gly);

(3) "region 3," Amino acid 141 (Asn) to amino acid 174 (Ala); and

(4) "region 4," the carboxy terminal hydrophilic region, amino acid 193 (Cys) to amino acid 261 (Val).

Within zsig28 are the several motifs of conserved amino acids based comparison amongst family

members (See Figure 1). Moreover, several regions of low variance are also present within zsig28 polypeptide. For determination of regions of low variance, see Sheppard, P. et al., Gene 150:163-167, 1994. Examining a multiple alignment of several known family members (For example, see Figure 1) revealed the following motifs that are both conserved and exhibit low degeneracy:

1) "motif 1" (a consensus motif pattern encompassing information in figure 1 that corresponds to amino acids 48 to 54 of SEQ ID NO:2);

2) "motif 2" (a consensus motif pattern encompassing information in figure 1 that corresponds to amino acids 77 to 82 of SEQ ID NO:2);

3) "motif 3" (a consensus motif pattern encompassing information in figure 1 that corresponds to amino acids 174 to 180 of SEQ ID NO:2);

Motifs 1 through 3 are spaced apart from N-terminus to C-terminus in a configuration represented by the following:

Met-{47-50}-M1-{21-22}-M2-{73-92}-M3,

where Met is the starting methionine residue

M# denotes the specific motif disclosed above (e.g., M1 is motif 1, etc.) and

{#} denotes the number of amino acids between the motifs.

In addition, another conserved motif in the third transmembrane domain of zsig28 is evident:

4) "motif 4" (a consensus motif pattern encompassing information in figure 1 that corresponds to amino acids 184 to 189 of SEQ ID NO:2);

Motifs 1 through 4 are spaced apart from N-terminus to C-terminus in a configuration represented by the following:

Met-{47-50}-M1-{21-22}-M2-{73-92}-M3-{3}-M4,

where Met is the starting methionine residue

where M# denotes the specific motif disclosed above (e.g., M4 is motif 4, etc.) and

{#} denotes the number of amino acids between the motifs.

The presence of transmembrane regions, and conserved and low variance motifs generally correlates with or defines important structural regions in proteins. Regions of low variance (e.g., hydrophobic clusters) are generally present in regions of structural importance (Sheppard, P. et al., supra.). Such regions of low variance often contain rare or infrequent amino acids, such as Tryptophan. The regions flanking and between such conserved and low variance motifs may be more variable, but are often functionally significant because they may relate to or define important structures and activities such as binding domains, biological and enzymatic activity, signal transduction, cell-cell interaction, tissue localization domains and the like. For example, regions 1 through 4 described above may be functionally significant.

In addition, there are several individual conserved amino acids throughout the zsig28 polypeptide located in SEQ ID NO:2 at the following amino acid numbers: 30 (Trp), 48 (Gly), 49 (Leu), 50 (Trp), 53 (Cys), 59 (Gly), 63 (Cys), 72 (Leu), 103 (Cys), and 114 (Lys).

The regions of conserved amino acid residues in zsig28, described above, can be used as tools to identify new family members. For instance, reverse transcription-polymerase chain reaction (RT-PCR) can be used to amplify sequences encoding the conserved regions from RNA obtained from a variety of tissue sources or cell lines. In particular, highly degenerate primers designed from the zsig28 sequences are useful for this purpose. Designing

and using such degenerate primers may be readily performed by one of skill in the art.

The corresponding polynucleotides encoding the zsig28 polypeptide regions, domains, motifs, residues and sequences described above are as shown in SEQ ID NO:1.

The present invention also provides polynucleotide molecules, including DNA and RNA molecules, that encode the zsig28 polypeptides disclosed herein. Those skilled in the art will readily recognize that, in view of the degeneracy of the genetic code, considerable sequence variation is possible among these polynucleotide molecules. SEQ ID NO:10 is a degenerate DNA sequence that encompasses all DNAs that encode the zsig28 polypeptide of SEQ ID NO:2. Those skilled in the art will recognize that the degenerate sequence of SEQ ID NO:10 also provides all RNA sequences encoding SEQ ID NO:2 by substituting U for T. Thus, zsig28 polypeptide-encoding polynucleotides comprising nucleotide 1 to nucleotide 783 of SEQ ID NO:10 and their RNA equivalents are contemplated by the present invention. Table 1 sets forth the one-letter codes used within SEQ ID NO:10 to denote degenerate nucleotide positions. "Resolutions" are the nucleotides denoted by a code letter. "Complement" indicates the code for the complementary nucleotide(s). For example, the code Y denotes either C or T, and its complement R denotes A or G, A being complementary to T, and G being complementary to C.

TABLE 1

Nucleotide	Resolution	Complement	Resolution
A	A	T	T
C	C	G	G

G	G	C	C
T	T	A	A
R	A|G	Y	C|T
Y	C|T	R	A|G
M	A|C	K	G|T
K	G|T	M	A|C
S	C|G	S	C|G
W	A|T	W	A|T
H	A|C|T	D	A|G|T
B	C|G|T	V	A|C|G
V	A|C|G	B	C|G|T
D	A|G|T	H	A|C|T
N	A|C|G|T	N	A|C|G|T

The degenerate codons used in SEQ ID NO:10, encompassing all possible codons for a given amino acid, are set forth in Table 2.

TABLE 2

Amino Acid	One Letter Code	Codons	Degenerate Codon
Cys	C	TGC TGT	TGY
Ser	S	AGC AGT TCA TCC TCG TCT	WSN
Thr	T	ACA ACC ACG ACT	ACN
Pro	P	CCA CCC CCG CCT	CCN
Ala	A	GCA GCC GCG GCT	GCN
Gly	G	GGA GGC GGG GGT	GGN
Asn	N	AAC AAT	AAY
Asp	D	GAC GAT	GAY
Glu	E	GAA GAG	GAR
Gln	Q	CAA CAG	CAR
His	H	CAC CAT	CAY
Arg	R	AGA AGG CGA CGC CGG CGT	MGN
Lys	K	AAA AAG	AAR
Met	M	ATG	ATG
Ile	I	ATA ATC ATT	ATH
Leu	L	CTA CTC CTG CTT TTA TTG	YTN
Val	V	GTA GTC GTG GTT	GTN
Phe	F	TTC TTT	TTY
Tyr	Y	TAC TAT	TAY
Trp	W	TGG	TGG
Ter	.	TAA TAG TGA	TRR
Asn|Asp	B		RAY
Glu|Gln	Z		SAR
Any	X		NNN

One of ordinary skill in the art will appreciate that some ambiguity is introduced in determining a degenerate codon, representative of all possible codons encoding each amino acid. For example, the degenerate codon for serine (WSN) can, in some circumstances, encode arginine (AGR), and the degenerate codon for arginine (MGN) can, in some circumstances, encode serine (AGY). A similar relationship exists between codons encoding phenylalanine and leucine. Thus, some polynucleotides encompassed by the degenerate sequence may encode variant amino acid sequences, but one of ordinary skill in the art can easily identify such variant sequences by reference to the amino acid sequence of SEQ ID NO:2. Variant sequences can be readily tested for functionality as described herein.

One of ordinary skill in the art will also appreciate that different species can exhibit "preferential codon usage." In general, see, Grantham, et al., Nuc. Acids Res. 8:1893-912, 1980; Haas, et al. Curr. Biol. 6:315-24, 1996; Wain-Hobson, et al., Gene 13:355-64, 1981; Grosjean and Fiers, Gene 18:199-209, 1982; Holm, Nuc. Acids Res. 14:3075-87, 1986; Ikemura, J. Mol. Biol. 158:573-97, 1982. As used herein, the term "preferential codon usage" or "preferential codons" is a term of art referring to protein translation codons that are most frequently used in cells of a certain species, thus favoring one or a few representatives of the possible codons encoding each amino acid (See Table 2). For example, the amino acid Threonine (Thr) may be encoded by ACA, ACC, ACG, or ACT, but in mammalian cells ACC is the most commonly used codon; in other species, for example, insect cells, yeast, viruses or bacteria, different Thr codons may be preferential. Preferential codons for a particular species can be introduced into the polynucleotides of the present invention by a variety of methods known in the art. Introduction of preferential codon sequences into recombinant DNA can, for example,

enhance production of the protein by making protein translation more efficient within a particular cell type or species. Therefore, the degenerate codon sequence disclosed in SEQ ID NO:10 serves as a template for optimizing expression of polynucleotides in various cell types and species commonly used in the art and disclosed herein. Sequences containing preferential codons can be tested and optimized for expression in various species, and tested for functionality as disclosed herein.

Within preferred embodiments of the invention the isolated polynucleotides will hybridize to similar sized regions of SEQ ID NO:1, or a sequence complementary thereto, under stringent conditions. In general, stringent conditions are selected to be about 5°C lower than the thermal melting point (T_m) for the specific sequence at a defined ionic strength and pH. The T_m is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Numerous equations for calculating T_m are known in the art, and are specific for DNA, RNA and DNA-RNA hybrids and polynucleotide probe sequences of varying length (see, for example, Sambrook *et al.*, Molecular Cloning: A Laboratory Manual, Second Edition (Cold Spring Harbor Press 1989); Ausubel *et al.*, (eds.), Current Protocols in Molecular Biology (John Wiley and Sons, Inc. 1987); Berger and Kimmel (eds.), Guide to Molecular Cloning Techniques, (Academic Press, Inc. 1987); and Wetmur, Crit. Rev. Biochem. Mol. Biol. 26:227 (1990)). Sequence analysis software such as OLIGO 6.0 (LSR; Long Lake, MN) and *Primer Premier 4.0* (Premier Biosoft International; Palo Alto, CA), as well as sites on the Internet, are available tools for analyzing a given sequence and calculating T_m based on user defined criteria. Such programs can also analyze a given sequence under

defined conditions and identify suitable probe sequences. Typically, hybridization of longer polynucleotide sequences (e.g., >50 base pairs) is performed at temperatures of about 20-25°C below the calculated T_m. For smaller probes (e.g., <50 base pairs) hybridization is typically carried out at the T_m or 5-10°C below. This allows for the maximum rate of hybridization for DNA-DNA and DNA-RNA hybrids. Higher degrees of stringency at lower temperatures can be achieved with the addition of formamide which reduces the T_m of the hybrid about 1°C for each 1% formamide in the buffer solution. Suitable stringent hybridization conditions are equivalent to about a 5 h to overnight incubation at about 42°C in a solution comprising: about 40-50% formamide, up to about 6X SSC, about 5X Denhardt's solution, zero up to about 10% dextran sulfate, and about 10-20 µg/ml denatured commercially-available carrier DNA. Generally, such stringent conditions include temperatures of 20-70°C and a hybridization buffer containing up to 6x SSC and 0-50% formamide; hybridization is then followed by washing filters in up to about 2X SSC. For example, a suitable wash stringency is equivalent to 0.1X SSC to 2X SSC, 0.1% SDS, at 55°C to 65°C. Different degrees of stringency can be used during hybridization and washing to achieve maximum specific binding to the target sequence. Typically, the washes following hybridization are performed at increasing degrees of stringency to remove non-hybridized polynucleotide probes from hybridized complexes. Stringent hybridization and wash conditions depend on the length of the probe, reflected in the Tm, hybridization and wash solutions used, and are routinely determined empirically by one of skill in the art.

As previously noted, the isolated polynucleotides of the present invention include DNA and

RNA. Methods for preparing DNA and RNA are well known in the art. In general, RNA is isolated from a tissue or cell that produces large amounts of zsig28 RNA. Such tissues and cells are identified by Northern blotting (Thomas, Proc. Natl. Acad. Sci. USA 77:5201, 1980), and include stomach and lung. Total RNA can be prepared using guanidinium isothiocyanate extraction followed by isolation by centrifugation in a CsCl gradient (Chirgwin et al., Biochemistry 18:52-94, 1979). Poly $(A)^+$ RNA is prepared from total RNA using the method of Aviv and Leder (Proc. Natl. Acad. Sci. USA 69:1408-12, 1972). Complementary DNA (cDNA) is prepared from poly$(A)^+$ RNA using known methods. In the alternative, genomic DNA can be isolated. Polynucleotides encoding zsig28 polypeptides are then identified and isolated by, for example, hybridization or polymerase chain reaction (PCR) (Mullis, U.S. Patent No. 4,683,202).

A full-length clone encoding zsig28 can be obtained by conventional cloning procedures. Complementary DNA (cDNA) clones are preferred, although for some applications (e.g., expression in transgenic animals) it may be preferable to use a genomic clone, or to modify a cDNA clone to include at least one genomic intron from the same or a different gene. Methods for preparing cDNA and genomic clones are well known and within the level of ordinary skill in the art, and include the use of the sequence disclosed herein, or parts thereof, for probing or priming a library. Expression libraries can be probed with antibodies to zsig28 polypeptide, receptor fragments, or other specific binding partners.

The polynucleotides of the present invention can also be synthesized using DNA synthesis machines. Currently the method of choice is the phosphoramidite method. If chemically synthesized double stranded DNA is required for an application such as the synthesis of a

gene or a gene fragment, then each complementary strand is made separately. The production of short polynucleotides (60 to 80 bp) is technically straightforward and can be accomplished by synthesizing the complementary strands and then annealing them. However, for producing longer polynucleotides (>300 bp), special strategies are usually employed, because the coupling efficiency of each cycle during chemical DNA synthesis is seldom 100%. To overcome this problem, synthetic genes (double-stranded) are assembled in modular form from single-stranded fragments that are from 20 to 100 nucleotides in length.

One method for building a synthetic gene requires the initial production of a set of overlapping, complementary oligonucleotides, each of which is between 20 to 60 nucleotides long. Each internal section of the gene has complementary 3' and 5' terminal extensions designed to base pair precisely with an adjacent section. Thus, after the gene is assembled, process is completed by sealing the nicks along the backbones of the two strands with T4 DNA ligase. In addition to the protein coding sequence, synthetic genes can be designed with terminal sequences that facilitate insertion into a restriction endonuclease site of a cloning vector. Moreover, other sequences should can be added that contain signals for proper initiation and termination of transcription and translation.

An alternative way to prepare a full-length gene is to synthesize a specified set of overlapping oligonucleotides (40 to 100 nucleotides). After the 3' and 5' short overlapping complementary regions (6 to 10 nucleotides) are annealed, large gaps still remain, but the short base-paired regions are both long enough and stable enough to hold the structure together. The are gaps filled and the DNA duplex is completed via enzymatic DNA

synthesis by *E. coli* DNA polymerase I. After the enzymatic synthesis is completed, the nicks are sealed with T4 DNA ligase. Double-stranded constructs are sequentially linked to one another to form the entire gene sequence which is verified by DNA sequence analysis. See Glick and Pasternak, Molecular Biotechnology, Principles & Applications of Recombinant DNA, (ASM Press, Washington, D.C. 1994); Itakura et al., Annu. Rev. Biochem. 53: 323-56, 1984 and Climie et al., Proc. Natl. Acad. Sci. USA 87:633-7, 1990.

zsig28 polynucleotide sequences disclosed herein can also be used as probes or primers to clone 5' non-coding regions of a zsig28 gene. In view of the tissue-specific expression observed for zsig28 by Northern blotting, this gene region is expected to provide for stomach-specific expression. Promoter elements from a zsig28 gene could thus be used to direct the tissue-specific expression of heterologous genes in, for example, transgenic animals or patients treated with gene therapy. Cloning of 5' flanking sequences also facilitates production of zsig28 proteins by "gene activation" as disclosed in U.S. Patent No. 5,641,670. Briefly, expression of an endogenous zsig28 gene in a cell is altered by introducing into the zsig28 locus a DNA construct comprising at least a targeting sequence, a regulatory sequence, an exon, and an unpaired splice donor site. The targeting sequence is a zsig28 5' non-coding sequence that permits homologous recombination of the construct with the endogenous zsig28 locus, whereby the sequences within the construct become operably linked with the endogenous zsig28 coding sequence. In this way, an endogenous zsig28 promoter can be replaced or supplemented with other regulatory sequences to provide enhanced, tissue-specific, or otherwise regulated expression.

The present invention further provides counterpart polypeptides and polynucleotides from other

species (orthologs). These species include, but are not limited to mammalian, avian, amphibian, reptile, fish, insect and other vertebrate and invertebrate species. Of particular interest are zsig28 polypeptides from other mammalian species, including murine, porcine, ovine, bovine, canine, feline, equine, and other primate polypeptides. Orthologs of human zsig28 can be cloned using information and compositions provided by the present invention in combination with conventional cloning techniques. For example, a cDNA can be cloned using mRNA obtained from a tissue or cell type that expresses zsig28 as disclosed herein. Suitable sources of mRNA can be identified by probing Northern blots with probes designed from the sequences disclosed herein. A library is then prepared from mRNA of a positive tissue or cell line. A zsig28-encoding cDNA can then be isolated by a variety of methods, such as by probing with a complete or partial human cDNA or with one or more sets of degenerate probes based on the disclosed sequences. A cDNA can also be cloned using the polymerase chain reaction, or PCR (Mullis, supra.), using primers designed from the representative human zsig28sequence disclosed herein. Within an additional method, the cDNA library can be used to transform or transfect host cells, and expression of the cDNA of interest can be detected with an antibody to zsig28 polypeptide. Similar techniques can also be applied to the isolation of genomic clones.

Those skilled in the art will recognize that the sequence disclosed in SEQ ID NO:1 represents a single allele of human zsig28 and that allelic variation and alternative splicing are expected to occur. Allelic variants of this sequence can be cloned by probing cDNA or genomic libraries from different individuals according to standard procedures. Allelic variants of the DNA sequence shown in SEQ ID NO:1, including those containing silent mutations and those in which mutations result in

amino acid sequence changes, are within the scope of the present invention, as are proteins which are allelic variants of SEQ ID NO:2. cDNAs generated from alternatively spliced mRNAs, which retain the properties of the zsig28 polypeptide are included within the scope of the present invention, as are polypeptides encoded by such cDNAs and mRNAs. Allelic variants and splice variants of these sequences can be cloned by probing cDNA or genomic libraries from different individuals or tissues according to standard procedures known in the art.

The present invention also provides isolated zsig28 polypeptides that are substantially similar to the polypeptides of SEQ ID NO:2 and their orthologs. The term "substantially similar" is used herein to denote polypeptides having at least 70%, more preferably at least 80%, sequence identity to the sequences shown in SEQ ID NO:2 or their orthologs. Such polypeptides will more preferably be at least 90% identical, and most preferably 95% or more identical to SEQ ID NO:2 or its orthologs. Percent sequence identity is determined by conventional methods. See, for example, Altschul et al., Bull. Math. Bio. 48: 603-16, 1986 and Henikoff and Henikoff, Proc. Natl. Acad. Sci. USA 89:10915-9, 1992. Briefly, two amino acid sequences are aligned to optimize the alignment scores using a gap opening penalty of 10, a gap extension penalty of 1, and the "blosum 62" scoring matrix of Henikoff and Henikoff (ibid.) as shown in Table 3 (amino acids are indicated by the standard one-letter codes). The percent identity is then calculated as:

$$\frac{\text{Total number of identical matches}}{\text{[length of the longer sequence plus the number of gaps introduced into the longer sequence in order to align the two sequences]}} \times 100$$

Table 3

	A	R	N	D	C	Q	E	G	H	I	L	K	M	F	P	S	T	W	Y	V
A	4																			
R	-1	5																		
N	-2	0	6																	
D	-2	-2	1	6																
C	0	-3	-3	-3	9															
Q	-1	1	0	0	-3	5														
E	-1	0	0	2	-4	2	5													
G	0	-2	0	-1	-3	-2	-2	6												
H	-2	0	1	-1	-3	0	0	-2	8											
I	-1	-3	-3	-3	-1	-3	-3	-4	-3	4										
L	-1	-2	-3	-4	-1	-2	-3	-4	-3	2	4									
K	-1	2	0	-1	-3	1	1	-2	-1	-3	-2	5								
M	-1	-1	-2	-3	-1	0	-2	-3	-2	1	2	-1	5							
F	-2	-3	-3	-3	-2	-3	-3	-3	-1	0	0	-3	0	6						
P	-1	-2	-2	-1	-3	-1	-1	-2	-2	-3	-3	-1	-2	-4	7					
S	1	-1	1	0	-1	0	0	0	-1	-2	-2	0	-1	-2	-1	4				
T	0	-1	0	-1	-1	-1	-1	-2	-2	-1	-1	-1	-1	-2	-1	1	5			
W	-3	-3	-4	-4	-2	-2	-3	-2	-2	-3	-2	-3	-1	1	-4	-3	-2	11		
Y	-2	-2	-2	-3	-2	-1	-2	-3	2	-1	-1	-2	-1	3	-3	-2	-2	2	7	
V	0	-3	-3	-3	-1	-2	-2	-3	-3	3	1	-2	1	-1	-2	-2	0	-3	-1	4

Sequence identity of polynucleotide molecules is determined by similar methods using a ratio as disclosed above.

Those skilled in the art appreciate that there are many established algorithms available to align two amino acid sequences. The "FASTA" similarity search algorithm of Pearson and Lipman is a suitable protein alignment method for examining the level of identity shared by an amino acid sequence disclosed herein and the amino acid sequence of a putative variant zsig28 polypeptide. The FASTA algorithm is described by Pearson and Lipman, *Proc. Nat'l Acad. Sci. USA 85*:2444 (1988), and by Pearson, *Meth. Enzymol. 183*:63 (1990).

Briefly, FASTA first characterizes sequence similarity by identifying regions shared by the query sequence (*e.g.*, SEQ ID NO:2) and a test sequence that have either the highest density of identities (if the ktup variable is 1) or pairs of identities (if ktup=2), without considering conservative amino acid substitutions, insertions, or deletions. The ten regions with the highest density of identities are then rescored by comparing the similarity of all paired amino acids using an amino acid substitution matrix, and the ends of the regions are "trimmed" to include only those residues that contribute to the highest score. If there are several regions with scores greater than the "cutoff" value (calculated by a predetermined formula based upon the length of the sequence and the ktup value), then the trimmed initial regions are examined to determine whether the regions can be joined to form an approximate alignment with gaps. Finally, the highest scoring regions of the two amino acid sequences are aligned using a modification of the Needleman-Wunsch-Sellers algorithm (Needleman and Wunsch, *J. Mol. Biol. 48*:444 (1970); Sellers, *SIAM J. Appl. Math. 26*:787 (1974)), which allows for amino acid insertions and deletions. Preferred parameters for FASTA

analysis are: ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62. These parameters can be introduced into a FASTA program by modifying the scoring matrix file ("SMATRIX"), as explained in Appendix 2 of Pearson, *Meth. Enzymol. 183*:63 (1990).

FASTA can also be used to determine the sequence identity of nucleic acid molecules using a ratio as disclosed above. For nucleotide sequence comparisons, the ktup value can range between one to six, preferably from three to six, most preferably three, with other parameters set as default.

The BLOSUM62 table (Table 3) is an amino acid substitution matrix derived from about 2,000 local multiple alignments of protein sequence segments, representing highly conserved regions of more than 500 groups of related proteins (Henikoff and Henikoff, Proc. Nat'l Acad. Sci. USA 89:10915 (1992)). Accordingly, the BLOSUM62 substitution frequencies can be used to define conservative amino acid substitutions that may be introduced into the amino acid sequences of the present invention. Although it is possible to design amino acid substitutions based solely upon chemical properties (as discussed below), the language "conservative amino acid substitution" preferably refers to a substitution represented by a BLOSUM62 value of greater than -1. For example, an amino acid substitution is conservative if the substitution is characterized by a BLOSUM62 value of 0, 1, 2, or 3. According to this system, preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 1 (e.g., 1, 2 or 3), while more preferred conservative amino acid substitutions are characterized by a BLOSUM62 value of at least 2 (e.g., 2 or 3).

Variant zsig28 polypeptides or substantially homologous zsig28 polypeptides are characterized as having one or more amino acid substitutions, deletions or additions. These changes are preferably of a minor nature, that is conservative amino acid substitutions (see Table 4) and other substitutions that do not significantly affect the folding or activity of the polypeptide; small deletions, typically of one to about 30 amino acids; and amino- or carboxyl-terminal extensions, such as an amino-terminal methionine residue, a small linker peptide of up to about 20-25 residues, or an affinity tag. The present invention thus includes polypeptides of from about 208 to about 291 amino acid residues that comprise a sequence that is at least 80%, preferably at least 90%, and more preferably 95% or more identical to the corresponding region of SEQ ID NO:2. Polypeptides comprising affinity tags can further comprise a proteolytic cleavage site between the zsig28 polypeptide and the affinity tag. Suitable sites include thrombin cleavage sites and factor Xa cleavage sites.

Table 4

Conservative amino acid substitutions

Basic:	arginine
	lysine
	histidine
Acidic:	glutamic acid
	aspartic acid
Polar:	glutamine
	asparagine
Hydrophobic:	leucine
	isoleucine
	valine
Aromatic:	phenylalanine
	tryptophan

	tyrosine
Small:	glycine
	alanine
	serine
	threonine
	methionine

The present invention further provides a variety of other polypeptide fusions and related multimeric proteins comprising one or more polypeptide fusions. For example, a zsig28 polypeptide can be prepared as a fusion to a dimerizing protein as disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Preferred dimerizing proteins in this regard include immunoglobulin constant region domains. Immunoglobulin-zsig28 polypeptide fusions can be expressed in genetically engineered cells to produce a variety of multimeric zsig28 analogs. Auxiliary domains can be fused to zsig28 polypeptides to target them to specific cells, tissues, or macromolecules (e.g., collagen). For example, a zsig28 polypeptide or protein could be targeted to a predetermined cell type by fusing a zsig28 polypeptide to a ligand that specifically binds to a receptor on the surface of the target cell. In this way, polypeptides and proteins can be targeted for therapeutic or diagnostic purposes. A zsig28 polypeptide can be fused to two or more moieties, such as an affinity tag for purification and a targeting or dimerizing domain. Polypeptide fusions can also comprise one or more cleavage sites, particularly between domains. See, Tuan et al., Connective Tissue Research 34:1-9, 1996. Similarly, such fusions can be constructed to enable the secretion of the zsig28 polypeptide regions 1, 2, 3 or 4, described herein, or smaller fragments within those regions. Soluble zsig28 regions 1, 2, 3 or 4 attached to dimerizing proteins have can serve as antagonists of the natural ligand for zsig28 polypeptide, or to zsig28 polypeptide itself, for example

by preventing dimerization or multimerization. Such antagonists containing soluble zsig28 regions 1, 2, 3 or 4 can be tested for functionality as disclosed herein.

The proteins of the present invention can also comprise non-naturally occurring amino acid residues. Non-naturally occurring amino acids include, without limitation, *trans*-3-methylproline, 2,4-methanoproline, *cis*-4-hydroxyproline, *trans*-4-hydroxyproline, *N*-methylglycine, *allo*-threonine, methylthreonine, hydroxyethylcysteine, hydroxyethylhomocysteine, nitroglutamine, homoglutamine, pipecolic acid, thiazolidine carboxylic acid, dehydroproline, 3- and 4-methylproline, 3,3-dimethylproline, *tert*-leucine, norvaline, 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, and 4-fluorophenylalanine. Several methods are known in the art for incorporating non-naturally occurring amino acid residues into proteins. For example, an *in vitro* system can be employed wherein nonsense mutations are suppressed using chemically aminoacylated suppressor tRNAs. Methods for synthesizing amino acids and aminoacylating tRNA are known in the art. Transcription and translation of plasmids containing nonsense mutations is carried out in a cell-free system comprising an *E. coli* S30 extract and commercially available enzymes and other reagents. Proteins are purified by chromatography. See, for example, Robertson et al., J. Am. Chem. Soc. 113:2722, 1991; Ellman et al., Methods Enzymol. 202:301, 1991; Chung et al., Science 259:806-9, 1993; and Chung et al., Proc. Natl. Acad. Sci. USA 90:10145-9, 1993). In a second method, translation is carried out in *Xenopus* oocytes by microinjection of mutated mRNA and chemically aminoacylated suppressor tRNAs (Turcatti et al., J. Biol. Chem. 271:19991-8, 1996). Within a third method, *E. coli* cells are cultured in the absence of a natural amino acid that is to be replaced

(e.g., phenylalanine) and in the presence of the desired non-naturally occurring amino acid(s) (e.g., 2-azaphenylalanine, 3-azaphenylalanine, 4-azaphenylalanine, or 4-fluorophenylalanine). The non-naturally occurring amino acid is incorporated into the protein in place of its natural counterpart. See, Koide et al., Biochem. 33:7470-6, 1994. Naturally occurring amino acid residues can be converted to non-naturally occurring species by *in vitro* chemical modification. Chemical modification can be combined with site-directed mutagenesis to further expand the range of substitutions (Wynn and Richards, Protein Sci. 2:395-403, 1993).

A limited number of non-conservative amino acids, amino acids that are not encoded by the genetic code, non-naturally occurring amino acids, and unnatural amino acids may be substituted for zsig28 amino acid residues.

Essential amino acids in the polypeptides of the present invention can be identified according to procedures known in the art, such as site-directed mutagenesis or alanine-scanning mutagenesis (Cunningham and Wells, Science 244: 1081-5, 1989; Bass et al., Proc. Natl. Acad. Sci. USA 88:4498-502, 1991). In the latter technique, single alanine mutations are introduced at every residue in the molecule, and the resultant mutant molecules are tested for biological activity as disclosed below to identify amino acid residues that are critical to the activity of the molecule. See also, Hilton et al., J. Biol. Chem. 271:4699-708, 1996. Sites of protein-protein or other biological interaction can also be determined by physical analysis of structure, as determined by such techniques as nuclear magnetic resonance, crystallography, electron diffraction or photoaffinity labeling, in conjunction with mutation of putative contact site amino acids. See, for example, de Vos et al., Science 255:306-12, 1992; Smith et al., J. Mol. Biol. 224:899-904, 1992;

Wlodaver et al., FEBS Lett. 309:59-64, 1992. The identities of essential amino acids can also be inferred from analysis of homologies with related proteins, such as claudin 1 (SEQ ID NO:3), human OSP-like protein (SEQ ID NO:5), and the like.

Multiple amino acid substitutions can be made and tested using known methods of mutagenesis and screening, such as those disclosed by Reidhaar-Olson and Sauer (Science 241:53-7, 1988) or Bowie and Sauer (Proc. Natl. Acad. Sci. USA 86:2152-6, 1989). Briefly, these authors disclose methods for simultaneously randomizing two or more positions in a polypeptide, selecting for functional polypeptide, and then sequencing the mutagenized polypeptides to determine the spectrum of allowable substitutions at each position. Other methods that can be used include phage display (e.g., Lowman et al., Biochem. 30:10832-7, 1991; Ladner et al., U.S. Patent No. 5,223,409; Huse, WIPO Publication WO 92/06204) and region-directed mutagenesis (Derbyshire et al., Gene 46:145, 1986; Ner et al., DNA 7:127, 1988).

Variants of the disclosed zsig28 DNA and polypeptide sequences can be generated through DNA shuffling as disclosed by Stemmer, Nature 370:389-91, 1994, Stemmer, Proc. Natl. Acad. Sci. USA 91:10747-51, 1994 and WIPO Publication WO 97/20078. Briefly, variant DNAs are generated by *in vitro* homologous recombination by random fragmentation of a parent DNA followed by reassembly using PCR, resulting in randomly introduced point mutations. This technique can be modified by using a family of parent DNAs, such as allelic variants or DNAs from different species, to introduce additional variability into the process. Selection or screening for the desired activity, followed by additional iterations of mutagenesis and assay provides for rapid "evolution" of sequences by selecting for desirable mutations while simultaneously selecting against detrimental changes.

Mutagenesis methods as disclosed herein can be combined with high-throughput, automated screening methods to detect activity of cloned, mutagenized polypeptides in host cells. Mutagenized DNA molecules that encode active polypeptides (e.g., signal transduction, or binding activities) can be recovered from the host cells and rapidly sequenced using modern equipment. These methods allow the rapid determination of the importance of individual amino acid residues in a polypeptide of interest, and can be applied to polypeptides of unknown structure.

Using the methods discussed herein, one of ordinary skill in the art can identify and/or prepare a variety of polypeptide fragments or variants of SEQ ID NO:2 or that retain, for example, binding, cell-cell communication, or signal transduction activity of the wild-type zsig28 protein. For example, using the methods described above, one could identify a ligand binding domain on zsig28; heterodimeric and homodimeric binding domains; other functional or structural domains; or other domains important for protein-protein interactions, cell-cell interactions, or signal transduction. Such polypeptides may also include additional polypeptide segments, such as affinity tags, as generally disclosed herein.

For any zsig28 polypeptide, including variants and fusion proteins, one of ordinary skill in the art can readily generate a fully degenerate polynucleotide sequence encoding that variant using the information set forth in Tables 1 and 2 above.

The zsig28 polypeptides of the present invention, including full-length polypeptides, biologically active fragments, and fusion polypeptides, can be produced in genetically engineered host cells according to conventional techniques. Suitable host cells

are those cell types that can be transformed or transfected with exogenous DNA and grown in culture, and include bacteria, fungal cells, and cultured higher eukaryotic cells. Eukaryotic cells, particularly cultured cells of multicellular organisms, are preferred. Techniques for manipulating cloned DNA molecules and introducing exogenous DNA into a variety of host cells are disclosed by Sambrook et al., Molecular Cloning: A Laboratory Manual, 2nd ed., Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1989, and Ausubel et al., eds., Current Protocols in Molecular Biology, John Wiley and Sons, Inc., NY, 1987.

In general, a DNA sequence encoding a zsig28 polypeptide is operably linked to other genetic elements required for its expression, generally including a transcription promoter and terminator, within an expression vector. Generally, the promoter is operably linked to the DNA sequence or segment, and the DNA segment is operably linked to the transcription terminator. The vector will also commonly contain one or more selectable markers and one or more origins of replication, although those skilled in the art will recognize that within certain systems selectable markers may be provided on separate vectors, and replication of the exogenous DNA may be provided by integration into the host cell genome. Selection of promoters, terminators, selectable markers, vectors and other elements is a matter of routine design within the level of ordinary skill in the art. Many such elements are described in the literature and are available through commercial suppliers.

To direct a zsig28 polypeptide into the secretory pathway of a host cell, a secretory signal sequence (also known as a leader sequence, prepro sequence or pre sequence) is provided in the expression vector. The secretory signal sequence may be that of zsig28, or may be derived from another secreted protein (e.g., t-PA)

or synthesized *de novo*. The secretory signal sequence is operably linked to the zsig28 DNA sequence, i.e., the two sequences are joined in the correct reading frame and positioned to direct the newly synthesized polypeptide into the secretory pathway of the host cell. Secretory signal sequences are commonly positioned 5' to the DNA sequence encoding the polypeptide of interest, although certain secretory signal sequences may be positioned elsewhere in the DNA sequence of interest (See, e.g., Welch et al., U.S. Patent No. 5,037,743; Holland et al., U.S. Patent No. 5,143,830).

Alternatively, the secretory signal sequence contained in the polypeptides of the present invention is used to direct other polypeptides into the secretory pathway. The present invention provides for such fusion polypeptides. A signal fusion polypeptide can be made wherein a secretory signal sequence derived from amino acid residue 1 (Met) to residue 23 (Ala) of SEQ ID NO:2 is operably linked to a DNA sequence encoding another polypeptide using methods known in the art and disclosed herein. The secretory signal sequence contained in the fusion polypeptides of the present invention is preferably fused amino-terminally to an additional peptide to direct the additional peptide into the secretory pathway. Such constructs have numerous applications known in the art. For example, these novel secretory signal sequence fusion constructs can direct the secretion of an active component of a normally non-secreted protein. Such fusions may be used *in vivo* or *in vitro* to direct peptides through the secretory pathway.

Cultured mammalian cells are suitable hosts within the present invention. Methods for introducing exogenous DNA into mammalian host cells include calcium phosphate-mediated transfection (Wigler et al., Cell 14:725, 1978; Corsaro and Pearson, Somatic Cell Genetics 7:603, 1981: Graham and Van der Eb, Virology 52:456,

1973), electroporation (Neumann et al., EMBO J. 1:841-5, 1982), DEAE-dextran mediated transfection (Ausubel et al., ibid.); and liposome-mediated transfection (Hawley-Nelson et al., Focus 15:73, 1993; Ciccarone et al., Focus 15:80, 1993, and viral vectors (Miller and Rosman, BioTechniques 7:980-90, 1989; Wang and Finer, Nature Med. 2:714-6, 1996). The production of recombinant polypeptides in cultured mammalian cells is disclosed, for example, by Levinson et al., U.S. Patent No. 4,713,339; Hagen et al., U.S. Patent No. 4,784,950; Palmiter et al., U.S. Patent No. 4,579,821; and Ringold, U.S. Patent No. 4,656,134. Suitable cultured mammalian cells include the COS-1 (ATCC No. CRL 1650), COS-7 (ATCC No. CRL 1651), BHK (ATCC No. CRL 1632), BHK 570 (ATCC No. CRL 10314), 293 (ATCC No. CRL 1573; Graham et al., J. Gen. Virol. 36:59-72, 1977) and Chinese hamster ovary (e.g. CHO-K1; ATCC No. CCL 61) cell lines. Additional suitable cell lines are known in the art and available from public depositories such as the American Type Culture Collection, Manassas, VA. In general, strong transcription promoters are preferred, such as promoters from SV-40 or cytomegalovirus. See, e.g., U.S. Patent No. 4,956,288. Other suitable promoters include those from metallothionein genes (U.S. Patent Nos. 4,579,821 and 4,601,978) and the adenovirus major late promoter.

Drug selection is generally used to select for cultured mammalian cells into which foreign DNA has been inserted. Such cells are commonly referred to as "transfectants". Cells that have been cultured in the presence of the selective agent and are able to pass the gene of interest to their progeny are referred to as "stable transfectants." A preferred selectable marker is a gene encoding resistance to the antibiotic neomycin. Selection is carried out in the presence of a neomycin-type drug, such as G-418 or the like. Selection systems can also be used to increase the expression level of the gene of interest, a process referred to as

"amplification." Amplification is carried out by culturing transfectants in the presence of a low level of the selective agent and then increasing the amount of selective agent to select for cells that produce high levels of the products of the introduced genes. A preferred amplifiable selectable marker is dihydrofolate reductase, which confers resistance to methotrexate. Other drug resistance genes (e.g. hygromycin resistance, multi-drug resistance, puromycin acetyltransferase) can also be used. Alternative markers that introduce an altered phenotype, such as green fluorescent protein, or cell surface proteins such as CD4, CD8, Class I MHC, placental alkaline phosphatase may be used to sort transfected cells from untransfected cells by such means as FACS sorting or magnetic bead separation technology.

Other higher eukaryotic cells can also be used as hosts, including plant cells, insect cells and avian cells. The use of *Agrobacterium rhizogenes* as a vector for expressing genes in plant cells has been reviewed by Sinkar et al., J. Biosci. (Bangalore) 11:47-58, 1987. Transformation of insect cells and production of foreign polypeptides therein is disclosed by Guarino et al., U.S. Patent No. 5,162,222 and WIPO publication WO 94/06463. Insect cells can be infected with recombinant baculovirus, commonly derived from *Autographa californica nuclear polyhedrosis virus* (AcNPV). See, King, L.A. and Possee, R.D., The Baculovirus Expression System: A Laboratory Guide, London, Chapman & Hall; O'Reilly, D.R. et al., Baculovirus Expression Vectors: A Laboratory Manual, New York, Oxford University Press., 1994; and, Richardson, C. D., Ed., Baculovirus Expression Protocols. Methods in Molecular Biology, Totowa, NJ, Humana Press, 1995. The second method of making recombinant zsig28 baculovirus utilizes a transposon-based system described by Luckow (Luckow, V.A, et al., J Virol 67:4566-79, 1993). This system, which utilizes transfer vectors, is sold in the

Bac-to-Bac™ kit (Life Technologies, Rockville, MD). This system utilizes a transfer vector, pFastBac1™ (Life Technologies) containing a Tn7 transposon to move the DNA encoding the zsig28 polypeptide into a baculovirus genome maintained in E. coli as a large plasmid called a "bacmid." The pFastBac1™ transfer vector utilizes the AcNPV polyhedrin promoter to drive the expression of the gene of interest, in this case zsig28. However, pFastBac1™ can be modified to a considerable degree. The polyhedrin promoter can be removed and substituted with the baculovirus basic protein promoter (also known as Pcor, p6.9 or MP promoter) which is expressed earlier in the baculovirus infection, and has been shown to be advantageous for expressing secreted proteins. See, Hill-Perkins, M.S. and Possee, R.D., J. Gen. Virol. 71:971-6, 1990; Bonning, B.C. et al., J. Gen. Virol. 75:1551-6, 1994; and, Chazenbalk, G.D., and Rapoport, B., J. Biol. Chem. 270:1543-9, 1995. In such transfer vector constructs, a short or long version of the basic protein promoter can be used. Moreover, transfer vectors can be constructed which replace the native zsig28 secretory signal sequences with secretory signal sequences derived from insect proteins. For example, a secretory signal sequence from Ecdysteroid Glucosyltransferase (EGT), honey bee Melittin (Invitrogen, Carlsbad, CA), or baculovirus gp67 (PharMingen, San Diego, CA) can be used in constructs to replace the native zsig28 secretory signal sequence. In addition, transfer vectors can include an in-frame fusion with DNA encoding an epitope tag at the C- or N-terminus of the expressed zsig28 polypeptide, for example, a Glu-Glu epitope tag (Grussenmeyer, T. et al., Proc. Natl. Acad. Sci. 82:7952-4, 1985). Using a technique known in the art, a transfer vector containing zsig28 is transformed into E. coli, and screened for bacmids which contain an interrupted lacZ gene indicative of recombinant baculovirus. The bacmid DNA containing the recombinant

baculovirus genome is isolated, using common techniques, and used to transfect *Spodoptera frugiperda* cells, e.g. Sf9 cells. Recombinant virus that expresses zsig28 is subsequently produced. Recombinant viral stocks are made by methods commonly used the art.

The recombinant virus is used to infect host cells, typically a cell line derived from the fall armyworm, *Spodoptera frugiperda*. See, in general, Glick and Pasternak, Molecular Biotechnology: Principles and Applications of Recombinant DNA, ASM Press, Washington, D.C., 1994. Another suitable cell line is the High FiveO™ cell line (Invitrogen) derived from *Trichoplusia ni* (U.S. Patent No. 5,300,435). Commercially available serum-free media are used to grow and maintain the cells. Suitable media are Sf900 II™ (Life Technologies) or ESF 921™ (Expression Systems) for the Sf9 cells; and Ex-cell0405™ (JRH Biosciences, Lenexa, KS) or Express FiveO™ (Life Technologies) for the *T. ni* cells. The cells are grown up from an inoculation density of approximately 2-5 x 10^5 cells to a density of 1-2 x 10^6 cells at which time a recombinant viral stock is added at a multiplicity of infection (MOI) of 0.1 to 10, more typically near 3. Procedures used are generally described in available laboratory manuals (King, L. A. and Possee, R.D., ibid.; O'Reilly, D.R. et al., ibid.; Richardson, C. D., ibid.). Subsequent purification of the zsig28 polypeptide from the supernatant can be achieved using methods described herein.

Fungal cells, including yeast cells, can also be used within the present invention. Yeast species of particular interest in this regard include *Saccharomyces cerevisiae*, *Pichia pastoris*, and *Pichia methanolica*. Methods for transforming *S. cerevisiae* cells with exogenous DNA and producing recombinant polypeptides

therefrom are disclosed by, for example, Kawasaki, U.S. Patent No. 4,599,311; Kawasaki et al., U.S. Patent No. 4,931,373; Brake, U.S. Patent No. 4,870,008; Welch et al., U.S. Patent No. 5,037,743; and Murray et al., U.S. Patent No. 4,845,075. Transformed cells are selected by phenotype determined by the selectable marker, commonly drug resistance or the ability to grow in the absence of a particular nutrient (e.g., leucine). A preferred vector system for use in *Saccharomyces cerevisiae* is the *POT1* vector system disclosed by Kawasaki et al. (U.S. Patent No. 4,931,373), which allows transformed cells to be selected by growth in glucose-containing media. Suitable promoters and terminators for use in yeast include those from glycolytic enzyme genes (see, e.g., Kawasaki, U.S. Patent No. 4,599,311; Kingsman et al., U.S. Patent No. 4,615,974; and Bitter, U.S. Patent No. 4,977,092) and alcohol dehydrogenase genes. See also U.S. Patents Nos. 4,990,446; 5,063,154; 5,139,936 and 4,661,454. Transformation systems for other yeasts, including *Hansenula polymorpha, Schizosaccharomyces pombe, Kluyveromyces lactis, Kluyveromyces fragilis, Ustilago maydis, Pichia pastoris, Pichia methanolica, Pichia guillermondii* and *Candida maltosa* are known in the art. See, for example, Gleeson et al., J. Gen. Microbiol. 132:3459-65, 1986 and Cregg, U.S. Patent No. 4,882,279. *Aspergillus* cells may be utilized according to the methods of McKnight et al., U.S. Patent No. 4,935,349. Methods for transforming *Acremonium chrysogenum* are disclosed by Sumino et al., U.S. Patent No. 5,162,228. Methods for transforming *Neurospora* are disclosed by Lambowitz, U.S. Patent No. 4,486,533.

The use of *Pichia methanolica* as host for the production of recombinant proteins is disclosed in WIPO Publications WO 97/17450, WO 97/17451, WO 98/02536, and WO 98/02565. DNA molecules for use in transforming *P.*

methanolica will commonly be prepared as double-stranded, circular plasmids, which are preferably linearized prior to transformation. For polypeptide production in *P. methanolica*, it is preferred that the promoter and terminator in the plasmid be that of a *P. methanolica* gene, such as a *P. methanolica* alcohol utilization gene (*AUG1* or *AUG2*). Other useful promoters include those of the dihydroxyacetone synthase (DHAS), formate dehydrogenase (FMD), and catalase (CAT) genes. To facilitate integration of the DNA into the host chromosome, it is preferred to have the entire expression segment of the plasmid flanked at both ends by host DNA sequences. A preferred selectable marker for use in *Pichia methanolica* is a *P. methanolica ADE2* gene, which encodes phosphoribosyl-5-aminoimidazole carboxylase (AIRC; EC 4.1.1.21), which allows *ade2* host cells to grow in the absence of adenine. For large-scale, industrial processes where it is desirable to minimize the use of methanol, it is preferred to use host cells in which both methanol utilization genes (*AUG1* and *AUG2*) are deleted. For production of secreted proteins, host cells deficient in vacuolar protease genes (*PEP4* and *PRB1*) are preferred. Electroporation is used to facilitate the introduction of a plasmid containing DNA encoding a polypeptide of interest into *P. methanolica* cells. It is preferred to transform *P. methanolica* cells by electroporation using an exponentially decaying, pulsed electric field having a field strength of from 2.5 to 4.5 kV/cm, preferably about 3.75 kV/cm, and a time constant (τ) of from 1 to 40 milliseconds, most preferably about 20 milliseconds.

Prokaryotic host cells, including strains of the bacteria *Escherichia coli*, *Bacillus* and other genera are also useful host cells within the present invention. Techniques for transforming these hosts and expressing

foreign DNA sequences cloned therein are well known in the art (see, e.g., Sambrook et al., ibid.). When expressing a zsig28 polypeptide in bacteria such as *E. coli*, the polypeptide may be retained in the cytoplasm, typically as insoluble granules, or may be directed to the periplasmic space by a bacterial secretion sequence. In the former case, the cells are lysed, and the granules are recovered and denatured using, for example, guanidine isothiocyanate or urea. The denatured polypeptide can then be refolded and dimerized by diluting the denaturant, such as by dialysis against a solution of urea and a combination of reduced and oxidized glutathione, followed by dialysis against a buffered saline solution. In the latter case, the polypeptide can be recovered from the periplasmic space in a soluble and functional form by disrupting the cells (by, for example, sonication or osmotic shock) to release the contents of the periplasmic space and recovering the protein, thereby obviating the need for denaturation and refolding.

Transformed or transfected host cells are cultured according to conventional procedures in a culture medium containing nutrients and other components required for the growth of the chosen host cells. A variety of suitable media, including defined media and complex media, are known in the art and generally include a carbon source, a nitrogen source, essential amino acids, vitamins and minerals. Media may also contain such components as growth factors or serum, as required. The growth medium will generally select for cells containing the exogenously added DNA by, for example, drug selection or deficiency in an essential nutrient which is complemented by the selectable marker carried on the expression vector or co-transfected into the host cell. *P. methanolica* cells are cultured in a medium comprising adequate sources of carbon, nitrogen and trace nutrients at a temperature of about 25°C to 35°C. Liquid cultures are provided with sufficient aeration by conventional means, such as shaking

of small flasks or sparging of fermentors. A preferred culture medium for *P. methanolica* is YEPD (2% D-glucose, 2% Bacto™ Peptone (Difco Laboratories, Detroit, MI), 1% Bacto™ yeast extract (Difco Laboratories), 0.004% adenine and 0.006% L-leucine).

It is preferred to purify the polypeptides of the present invention to ≥80% purity, more preferably to ≥90% purity, even more preferably ≥95% purity, and particularly preferred is a pharmaceutically pure state, that is greater than 99.9% pure with respect to contaminating macromolecules, particularly other proteins and nucleic acids, and free of infectious and pyrogenic agents. Preferably, a purified polypeptide is substantially free of other polypeptides, particularly other polypeptides of animal origin.

Expressed recombinant zsig28 polypeptides (or chimeric zsig28 polypeptides) can be purified using fractionation and/or conventional purification methods and media. Ammonium sulfate precipitation and acid or chaotrope extraction may be used for fractionation of samples. Exemplary purification steps may include hydroxyapatite, size exclusion, FPLC and reverse-phase high performance liquid chromatography. Suitable chromatographic media include derivatized dextrans, agarose, cellulose, polyacrylamide, specialty silicas, and the like. PEI, DEAE, QAE and Q derivatives are preferred. Exemplary chromatographic media include those media derivatized with phenyl, butyl, or octyl groups, such as Phenyl-Sepharose FF (Pharmacia), Toyopearl butyl 650 (Toso Haas, Montgomeryville, PA), Octyl-Sepharose (Pharmacia) and the like; or polyacrylic resins, such as Amberchrom CG 71 (Toso Haas) and the like. Suitable solid supports include glass beads, silica-based resins, cellulosic resins, agarose beads, cross-linked agarose beads, polystyrene beads, cross-linked polyacrylamide resins and the like that are insoluble under the conditions in which

they are to be used. These supports may be modified with reactive groups that allow attachment of proteins by amino groups, carboxyl groups, sulfhydryl groups, hydroxyl groups and/or carbohydrate moieties. Examples of coupling chemistries include cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, hydrazide activation, and carboxyl and amino derivatives for carbodiimide coupling chemistries. These and other solid media are well known and widely used in the art, and are available from commercial suppliers. Methods for binding receptor polypeptides to support media are well known in the art. Selection of a particular method is a matter of routine design and is determined in part by the properties of the chosen support. See, for example, Affinity Chromatography: Principles & Methods, Pharmacia LKB Biotechnology, Uppsala, Sweden, 1988.

The polypeptides of the present invention can be isolated by exploitation of their biochemical, structural, and biological properties. For example, immobilized metal ion adsorption (IMAC) chromatography can be used to purify histidine-rich proteins, including those comprising polyhistidine tags. Briefly, a gel is first charged with divalent metal ions to form a chelate (Sulkowski, Trends in Biochem. 3:1-7, 1985). Histidine- rich proteins will be adsorbed to this matrix with differing affinities, depending upon the metal ion used, and will be eluted by competitive elution, lowering the pH, or use of strong chelating agents. Other methods of purification include purification of glycosylated proteins by lectin affinity chromatography and ion exchange chromatography (Methods in Enzymol., Vol. 182, "Guide to Protein Purification", M. Deutscher, (ed.), Acad. Press, San Diego, 1990, pp.529-39). Within additional embodiments of the invention, a fusion of the polypeptide of interest and an affinity tag (e.g., maltose-binding protein, an immunoglobulin domain) may be constructed to facilitate purification.

Moreover, using methods described in the art, polypeptide fusions, or hybrid zsig28 proteins, are constructed using regions or domains of the inventive zsig28 in combination with paralogs (e.g., human OSP-like protein), orthologs (e.g., murine OSP or RVP.1) or heterologous proteins (Sambrook et al., ibid.; Altschul et al., ibid.; Picard, Cur. Opin. Biology, 5:511-5, 1994, and references therein). These methods allow the determination of the biological importance of larger domains or regions in a polypeptide of interest. Such hybrids may alter reaction kinetics, binding, constrict or expand the substrate specificity, or alter tissue and cellular localization of a polypeptide, and can be applied to polypeptides of unknown structure.

Fusion proteins can be prepared by methods known to those skilled in the art by preparing each component of the fusion protein and chemically conjugating them. Alternatively, a polynucleotide encoding both components of the fusion protein in the proper reading frame can be generated using known techniques and expressed by the methods described herein. For example, part or all of a domain(s) conferring a biological function may be swapped between zsig28 of the present invention with the functionally equivalent domain(s) from another family member. Such domains include, but are not limited to, the secretory signal sequence, transmembrane domains, regions 1 through 4, and motifs 1 through 4, as described herein. Such fusion proteins would be expected to have a biological functional profile that is the same or similar to polypeptides of the present invention or proteins to which they are fused, depending on the fusion constructed. Moreover, such fusion proteins may exhibit other properties as disclosed herein.

Standard molecular biological and cloning techniques can be used to swap the desired domains between the zsig28 polypeptide and those to which they are fused.

Generally, a DNA segment that encodes a domain of interest, e.g., a domain described above, are operably linked in frame, and inserted into an appropriate expression vector, as described herein. Such fusion proteins can be expressed, isolated, and assayed for activity as described herein.

zsig28 polypeptides or fragments thereof may also be prepared through chemical synthesis. zsig28 polypeptides may be monomers or multimers; glycosylated or non-glycosylated; pegylated or non-pegylated; and may or may not include an initial methionine amino acid residue.

Polypeptides of the present invention can also be synthesized by exclusive solid phase synthesis, partial solid phase methods, fragment condensation or classical solution synthesis. Methods for synthesizing polypeptides are well known in the art. See, for example, Merrifield, J. Am. Chem. Soc. 85:2149, 1963; Kaiser et al., Anal. Biochem. 34:595, 1970. After the entire synthesis of the desired peptide on a solid support, the peptide-resin is with a reagent which cleaves the polypeptide from the resin and removes most of the side-chain protecting groups. Such methods are well established in the art.

The activity of molecules of the present invention can be measured using a variety of assays that measure proliferation and/or differentiation of specific cell types, chemotaxis, adhesion, changes in ion channel influx, pH flux, regulation of second messenger levels and neurotransmitter release, cell motility, protein binding, apoptosis, or the like. Such assays are well known in the art. See, for example, in "Basic & Clinical Endocrinology Ser., Vol. Vol. 3," Cytochemical Bioassays: Techniques & Applications, Chayen; Chayen, Bitensky, eds., Dekker, New York, 1983.

The activity of molecules of the present invention can be measured using a variety of assays that

measure stimulation of gastrointestinal cell contractility, peristalsis, modulation of nutrient uptake and/or secretion of digestive enzymes. Of particular interest are changes in contractility of smooth muscle cells. For example, the contractile response of segments of mammalian duodenum or other gastrointestinal smooth muscle tissue (Depoortere et al., J. Gastrointestinal Motility 1:150-159, 1989, incorporated herein by reference). An exemplary *in vivo* assay uses an ultrasonic micrometer to measure the dimensional changes radially between commissures and longitudinally to the plane of the valve base (Hansen et al., Society of Thoracic Surgeons 60:S384-390, 1995).

Gastric motility is generally measured in the clinical setting as the time required for gastric emptying and subsequent transit time through the gastrointestinal tract. Gastric emptying scans are well known to those skilled in the art, and briefly, comprise use of an oral contrast agent, such as barium, or a radiolabeled meal. Solids and liquids can be measured independently. A test food or liquid is radiolabeled with an isotope (e.g. ^{99m}Tc), and after ingestion or administration, transit time through the gastrointestinal tract and gastric emptying are measured by visualization using gamma cameras (Meyer et al., Am. J. Dig. Dis. 21:296, 1976; Collins et al., Gut 24:1117, 1983; Maughan et al., Diabet. Med. 13 9 Supp. 5:S6-10, 1996 and Horowitz et al., Arch. Intern. Med. 145:1467-1472, 1985). These studies may be performed before and after the administration of a promotility agent to quantify the efficacy of the drug. Moreover, these assays can be used to test *in vivo* zsig28 agonists and antagonists, discussed below.

High expression of zsig28 polypeptide in the stomach suggests that modulators of zsig28 activity would be therapeutically useful. Such modulators could be agonists or antagonists that respectively stimulate or

inhibit zsig28 polypeptide activity. Effects of modulating zsig28 activity can be assayed by methods well known in the art.

Zsig28 agonists or antagonists thereof may be therapeutically useful for promoting wound healing, for example, in the stomach. To verify the presence of modulators of zsig28 polypeptides with these capabilities, agonists or antagonists of the present invention are evaluated with respect to their ability to facilitate wound healing according to procedures known in the art. If desired, zsig28 polypeptide performance in this regard can be compared to growth factor receptors, such as those for EGF, NGF, TGF-α, TGF-β, insulin, IGF-I, IGF-II, fibroblast growth factor (FGF) and the like. In addition, zsig28 polypeptide agonists or antagonists may be evaluated in combination with one or more growth factors to identify synergistic effects on zsig28 activity.

In addition, zsig28 agonists or antagonists may be therapeutically useful for anti-microbial applications. To verify the presence of modulators of zsig28 polypeptides with these capabilities, agonists or antagonists of the present invention are evaluated with respect to their antimicrobial properties according to procedures known in the art. See, for example, Barsum et al., Eur. Respir. J. 8(5): 709-14, 1995; Sandovsky-Losica et al., J. Med. Vet. Mycol. (England) 28(4): 279-87, 1990; Mehentee et al., J. Gen. Microbiol (England) 135 (Pt. 8): 2181-8, 1989; Segal and Savage, J. Med. Vet. Mycol. 24: 477-479, 1986 and the like. In addition, zsig28 agonists or antagonists thereof may be evaluated in combination with one or more antimicrobial agents to identify synergistic effects on modulating the zsig28 polypeptide.

Zsig28 polypeptide or fragments thereof can act as anti-microbial agents to block a pathogenic organism

for adhering to zsig28 receptor or as a bacterial toxin sink. Such anti-microbial agents operating via membrane association or pore forming mechanisms of action directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective substances or the cell wall/membrane thereof. Anti-microbial agents, capable of inhibiting microorganism proliferation or action or of disrupting microorganism integrity by either mechanism set forth above, are useful in methods for preventing contamination in cell culture by microbes susceptible to that anti-microbial activity. Such techniques involve culturing cells in the presence of an effective amount of said zsig28 polypeptide, polypeptide fragment, or an agonist or antagonist thereof. Alternatively, such anti-microbial agents could directly attach to an offending bacterial toxin, described below, and effectively inactivate its toxicity.

The zsig28 polypeptides of the present invention may play a role in bacterial pathogenesis in human stomach and intestinal infections. Zsig28 shares homology with the *Clostridium perfringens* enterotoxin (CPE) receptor and related proteins which bind CPE (Katahira, J. et al., J. Cell Biol. 136:1239-1247, 1997;and, Katahira, J. et al., J. Biol. Chem. 272:26652-26658, 1997). Similarly, zsig28 may bind CPE or other bacterial enterotoxins or exotoxins, herein collectively described as "bacterial toxins." For example, such bacterial toxins are produced by bacteria that cause diseases such as food poisoning, Botulism, severe diarrhea, inflammation, cramping, or the like (e.g., *staphylococcus*, enterotoxigenic *E coli*, *campylobacter*, *Clostridium botulinum* and the like). Moreover, as a receptor, the zsig28 may be a site for the colonization and resultant pathogenic effects of

pathogenic bacteria in the stomach. For example, *Helicobacter pylori*, a causative agent in gastric ulcers, may exert its effects by binding to zsig28 and inducing cell lysis through apoptosis or other mechanisms. Such a role for zsig28 can be elucidated by one of skill in the art. For example, mammalian cells that are normally insensitive to a bacterial toxin can be transfected with zsig28 and tested for susceptibility to the bacterial toxin (See Katahira, J. et al., supra.; and, Katahira, J. et al., supra.;). When compared in parallel to untransfected cells, the susceptible zsig28-expressing cells can be measured by morphological changes associated with cell death such as (blebbing), lysis, and depletion of cell number when compared to the untransfected control. Alternatively, assays measuring direct binding of a radiolabeled or fluorescent labeled bacterial toxin can be employed to measure direct binding to zsig28-expressing cells. Similarly, using these assays disclosed above, whole bacterial cells can be tested for their ability to bind and or lyse cells expressing zsig28 polypeptide.

Thus, zsig28 polypeptides or agonists thereof may be used as cell culture reagents in *in vitro* studies of exogenous microorganism infection, such as bacterial, viral or fungal infection. Such moieties may also be used in *in vivo* animal models of infection. Also, the microorganism-adherence properties of zsig28 polypeptides or agonists thereof can be studied under a variety of conditions in binding assays and the like.

As a receptor, the activity of zsig28 polypeptide can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the Cytosensor™

Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H.M. et al., Science 257:1906-1912, 1992; Pitchford, S. et al., Meth. Enzymol. 228:84-108, 1997; Arimilli, S. et al., J. Immunol. Meth. 212:49-59, 1998; Van Liefde, I. Et al., Eur. J. Pharmacol. 346:87-95, 1998. The microphysiometer can be used for assaying eukaryotic, prokaryotic, adherent or non-adherent cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of the zsig28 polypeptide. Preferably, the microphysiometer is used to measure responses of a zsig28-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express zsig28 polypeptide. Zsig28-expressing eukaryotic cells comprise cells into which zsig28 has been transfected, as described herein, creating a cell that is responsive to zsig28-modulating stimuli, or are cells naturally expressing zsig28, such as zsig28-expressing cells derived from stomach tissue. Differences, measured by an increase or decrease in extracellular acidification, in the response of cells expressing zsig28, relative to a control, are a direct measurement of zsig28-modulated cellular responses. Moreover, such zsig28-modulated responses can be assayed under a variety of stimuli. Also, using the microphysiometer, there is provided a method of identifying agonists and antagonists of zsig28 polypeptide, comprising providing cells expressing a zsig28 polypeptide, culturing a first portion of the cells in the absence of a test compound, culturing a second

portion of the cells in the presence of a test compound, and detecting an increase or a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Antagonists and agonists, including the natural ligand for zsig28 polypeptide, can be rapidly identified using this method.

An *in vivo* approach for assaying proteins of the present invention involves viral delivery systems. Exemplary viruses for this purpose include adenovirus, herpesvirus, vaccinia virus, retroviruses, and adeno-associated virus (AAV). Adenovirus, a double-stranded DNA virus, is currently the best studied gene transfer vector for delivery of heterologous nucleic acid (for review, see T.C. Becker et al., Meth. Cell Biol. 43:161-89, 1994; and J.T. Douglas and D.T. Curiel, Science & Medicine 4:44-53, 1997). The adenovirus system offers several advantages: (i) adenovirus can accommodate relatively large DNA inserts; (ii) can be grown to high-titer; (iii) infect a broad range of mammalian cell types; and (iv) can be used with many different promoters including ubiquitous, tissue specific, and regulatable promoters. Also, because adenoviruses are stable in the bloodstream, they can be administered by intravenous injection.

Using adenovirus vectors where portions of the adenovirus genome are deleted, inserts are incorporated into the viral DNA by direct ligation or by homologous recombination with a co-transfected plasmid. In an exemplary system, the essential E1 gene has been deleted from the viral vector, and the virus will not replicate unless the E1 gene is provided by the host cell (the human 293 cell line is exemplary). When intravenously administered to intact animals, adenovirus primarily targets the liver. If the adenoviral delivery system has an E1 gene deletion, the virus cannot replicate in the

host cells. However, the host's tissue (e.g., liver) will express and process (and, if a secretory signal sequence is present, secrete) the heterologous protein. Secreted proteins will enter the circulation in the highly vascularized liver, and effects on the infected animal can be determined.

Moreover, adenoviral vectors containing various deletions of viral genes can be used in an attempt to reduce or eliminate immune responses to the vector. Such adenoviruses are E1 deleted, and in addition contain deletions of E2A or E4 (Lusky, M. et al., J. Virol. 72:2022-2032, 1998; Raper, S.E. et al., Human Gene Therapy 9:671-679, 1998). In addition, deletion of E2b is reported to reduce immune responses (Amalfitano, A. et al., J. Virol. 72:926-933, 1998). Moreover, by deleting the entire adenovirus genome, very large inserts of heterologous DNA can be accommodated. Generation of so called "gutless" adenoviruses where all viral genes are deleted are particularly advantageous for insertion of large inserts of heterologous DNA. For review, see Yeh, P. and Perricaudet, M., FASEB J. 11:615-623, 1997.

The adenovirus system can also be used for protein production *in vitro*. By culturing adenovirus-infected non-293 cells under conditions where the cells are not rapidly dividing, the cells can produce proteins for extended periods of time. For instance, BHK cells are grown to confluence in cell factories, then exposed to the adenoviral vector encoding the secreted protein of interest. The cells are then grown under serum-free conditions, which allows infected cells to survive for several weeks without significant cell division. Alternatively, adenovirus vector infected 293 cells can be grown as adherent cells or in suspension culture at relatively high cell density to produce significant

amounts of protein (See Garnier et al., Cytotechnol. 15:145-55, 1994). With either protocol, an expressed, secreted heterologous protein can be repeatedly isolated from the cell culture supernatant, lysate, or membrane fractions depending on the disposition of the expressed protein in the cell. Within the infected 293 cell production protocol, non-secreted proteins may also be effectively obtained.

As a receptor, the activation of zsig28 polypeptide can be measured by a silicon-based biosensor microphysiometer which measures the extracellular acidification rate or proton excretion associated with receptor binding and subsequent physiologic cellular responses. An exemplary device is the Cytosensor™ Microphysiometer manufactured by Molecular Devices, Sunnyvale, CA. A variety of cellular responses, such as cell proliferation, ion transport, energy production, inflammatory response, regulatory and receptor activation, and the like, can be measured by this method. See, for example, McConnell, H.M. et al., Science 257:1906-1912, 1992; Pitchford, S. et al., Meth. Enzymol. 228:84-108, 1997; Arimilli, S. et al., J. Immunol. Meth. 212:49-59, 1998; Van Liefde, I. Et al., Eur. J. Pharmacol. 346:87-95, 1998. The microphysiometer can be used for assaying adherent or non-adherent eukaryotic or prokaryotic cells. By measuring extracellular acidification changes in cell media over time, the microphysiometer directly measures cellular responses to various stimuli, including agonists, ligands, or antagonists of the zsig28 polypeptide. Preferably, the microphysiometer is used to measure responses of a zsig28-expressing eukaryotic cell, compared to a control eukaryotic cell that does not express zsig28 polypeptide. ZSIG28-expressing eukaryotic cells comprise cells into which zsig28 has been transfected, as described

herein, creating a cell that is responsive to zsig28-modulating stimuli; or cells naturally expressing zsig28, such as zsig28-expressing cells derived from stomach tissue. Differences, measured by a change in extracellular acidification, for example, an increase or diminution in the response of cells expressing zsig28, relative to a control, are a direct measurement of zsig28-modulated cellular responses. Moreover, such zsig28-modulated responses can be assayed under a variety of stimuli. Also, using the microphysiometer, there is provided a method of identifying agonists and antagonists of zsig28 polypeptide, comprising providing cells expressing a zsig28 polypeptide, culturing a first portion of the cells in the absence of a test compound, culturing a second portion of the cells in the presence of a test compound, and detecting a change, for example, an increase or diminution, in a cellular response of the second portion of the cells as compared to the first portion of the cells. The change in cellular response is shown as a measurable change extracellular acidification rate. Antagonists and agonists, including the natural ligand for zsig28 polypeptide, can be rapidly identified using this method.

A zsig28 polypeptide, or polypeptide fragment thereof, can be expressed as a fusion with an immunoglobulin heavy chain constant region, typically an F_c fragment, which contains two constant region domains and lacks the variable region. Methods for preparing such fusions are disclosed in U.S. Patents Nos. 5,155,027 and 5,567,584. Such fusions are typically secreted as multimeric molecules wherein the Fc portions are disulfide bonded to each other and two non-Ig polypeptides are arrayed in closed proximity to each other. Fusions of this type can be used to affinity purify ligand, as an *in vitro* assay tool. For use in assays, the chimeras are

bound to a support via the F_C region and used in an enzyme linked immunosorbent assay (ELISA) format.

A zsig28 polypeptide can also be used for purification of ligand that binds it. The zsig28 polypeptide or a ligand-binding polypeptide fragment thereof can be used. The polypeptide is immobilized on a solid support, such as beads of agarose, cross-linked agarose, glass, cellulosic resins, silica-based resins, polystyrene, cross-linked polyacrylamide, or like materials that are stable under the conditions of use. Methods for linking polypeptides to solid supports are known in the art, and include amine chemistry, cyanogen bromide activation, N-hydroxysuccinimide activation, epoxide activation, sulfhydryl activation, and hydrazide activation. The resulting medium will generally be configured in the form of a column, and fluids containing ligand are passed through the column one or more times to allow ligand to bind to the receptor polypeptide. The ligand is then eluted using changes in salt concentration, chaotropic agents (guanidine HCl), or pH to disrupt ligand-receptor binding.

An assay system that uses a ligand-binding receptor, such as zsig28, (or an antibody, one member of a complement/ anti-complement pair) or a binding fragment thereof, and a commercially available biosensor instrument (BIAcore, Pharmacia Biosensor, Piscataway, NJ) may be advantageously employed. Such receptor, antibody, member of a complement/anti-complement pair or fragment is immobilized onto the surface of a receptor chip. Use of this instrument is disclosed by Karlsson, J. Immunol. Methods 145:229-40, 1991 and Cunningham and Wells, J. Mol. Biol. 234:554-63, 1993. A receptor, antibody, member or fragment is covalently attached, using amine or sulfhydryl chemistry, to dextran fibers that are attached to gold film within the flow cell. A test sample is passed through the cell. If a ligand, epitope, or opposite

member of the complement/anti-complement pair is present in the sample, it will bind to the immobilized receptor, antibody or member, respectively, causing a change in the refractive index of the medium, which is detected as a change in surface plasmon resonance of the gold film. This system allows the determination of on- and off-rates, from which binding affinity can be calculated, and assessment of stoichiometry of binding.

Ligand-binding receptor polypeptides can also be used within other assay systems known in the art. Such systems include Scatchard analysis for determination of binding affinity (see Scatchard, Ann. NY Acad. Sci. 51: 660-72, 1949) and calorimetric assays (Cunningham et al., Science 253:545-48, 1991; Cunningham et al., Science 245:821-25, 1991).

Zsig28 polypeptides can also be used to prepare antibodies that bind to zsig28 epitopes, peptides or polypeptides. The zsig28 polypeptide or a fragment thereof serves as an antigen (immunogen) to inoculate an animal and elicit an immune response. One of skill in the art would recognize that antigenic, epitope-bearing polypeptides contain a sequence of at least 6, preferably at least 9, and more preferably at least 15 to about 30 contiguous amino acid residues of a zsig28 polypeptide (e.g., SEQ ID NO:2). Polypeptides comprising a larger portion of a zsig28 polypeptide, i.e., from 30 to 10 residues up to the entire length of the amino acid sequence are included. Antigens or immunogenic epitopes can also include attached tags, adjuvants and carriers, as described herein. Suitable antigens include the zsig28 polypeptide encoded by SEQ ID NO:2 from amino acid number 24 (Ala) to 261 (Val) of SEQ ID NO:2 or a contiguous 9 to 238 AA amino acid fragment thereof. Preferred peptides to use as antigens are regions 1, 2, 3 and 4 disclosed herein, and zsig28 hydrophilic peptides such as those

predicted by one of skill in the art from a hydrophobicity plot (See figure 2). Zsig28 hydrophilic peptides include peptides comprising amino acid sequences selected from the group consisting of: (1) amino acid number 245 (Ala) to amino acid number 250 (Glu) of SEQ ID NO:2; (2) amino acid number 234 (Asn) to amino acid number 239 (Lys) of SEQ ID NO:2; (3) amino acid number 202 (Glu) to amino acid number 207 (Lys) of SEQ ID NO:2; (4) amino acid number 254 (Lys) to amino acid number 259 (Asp) of SEQ ID NO:2; and (5) amino acid number 110 (Glu) to amino acid number 115 (Ala) of SEQ ID NO:2. In addition, conserved motifs, and variable regions between conserved motifs of zsig28 are suitable antigens. Antibodies from an immune response generated by inoculation of an animal with these antigens can be isolated and purified as described herein. Methods for preparing and isolating polyclonal and monoclonal antibodies are well known in the art. See, for example, Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995; Sambrook et al., Molecular Cloning: A Laboratory Manual, Second Edition, Cold Spring Harbor, NY, 1989; and Hurrell, J. G. R., Ed., Monoclonal Hybridoma Antibodies: Techniques and Applications, CRC Press, Inc., Boca Raton, FL, 1982.

As would be evident to one of ordinary skill in the art, polyclonal antibodies can be generated from inoculating a variety of warm-blooded animals such as horses, cows, goats, sheep, dogs, chickens, rabbits, mice, and rats with a zsig28 polypeptide or a fragment thereof. The immunogenicity of a zsig28 polypeptide may be increased through the use of an adjuvant, such as alum (aluminum hydroxide) or Freund's complete or incomplete adjuvant. Polypeptides useful for immunization also include fusion polypeptides, such as fusions of zsig28 or

a portion thereof with an immunoglobulin polypeptide or with maltose binding protein. The polypeptide immunogen may be a full-length molecule or a portion thereof. If the polypeptide portion is "hapten-like", such portion may be advantageously joined or linked to a macromolecular carrier (such as keyhole limpet hemocyanin (KLH), bovine serum albumin (BSA) or tetanus toxoid) for immunization.

As used herein, the term "antibodies" includes polyclonal antibodies, affinity-purified polyclonal antibodies, monoclonal antibodies, and antigen-binding fragments, such as $F(ab')_2$ and Fab proteolytic fragments. Genetically engineered intact antibodies or fragments, such as chimeric antibodies, Fv fragments, single chain antibodies and the like, as well as synthetic antigen-binding peptides and polypeptides, are also included. Non-human antibodies may be humanized by grafting non-human CDRs onto human framework and constant regions, or by incorporating the entire non-human variable domains (optionally "cloaking" them with a human-like surface by replacement of exposed residues, wherein the result is a "veneered" antibody). In some instances, humanized antibodies may retain non-human residues within the human variable region framework domains to enhance proper binding characteristics. Through humanizing antibodies, biological half-life may be increased, and the potential for adverse immune reactions upon administration to humans is reduced.

Moreover, human antibodies can be produced in transgenic, non-human animals that have been engineered to contain human immunoglobulin genes as disclosed in WIPO Publication WO 98/24893. It is preferred that the endogenous immunoglobulin genes in these animals be inactivated or eliminated, such as by homologous recombination.

Antibodies are considered to be specifically binding if: 1) they exhibit a threshold level of binding activity, and 2) they do not significantly cross-react with related polypeptide molecules. A threshold level of binding is determined if anti-zsig28 antibodies herein bind to a zsig28 polypeptide, peptide or epitope with an affinity at least 10-fold greater than the binding affinity to control (non-zsig28) polypeptide. It is preferred that the antibodies exhibit a binding affinity (K_a) of 10^6 M^{-1} or greater, preferably 10^7 M^{-1} or greater, more preferably 10^8 M^{-1} or greater, and most preferably 10^9 M^{-1} or greater. The binding affinity of an antibody can be readily determined by one of ordinary skill in the art, for example, by Scatchard analysis (Scatchard, G., Ann. NY Acad. Sci. 51: 660-672, 1949).

Whether anti-zsig28 antibodies do not significantly cross-react with related polypeptide molecules is shown, for example, by the antibody detecting zsig28 polypeptide but not known related polypeptides using a standard Western blot analysis (Ausubel et al., ibid.). Examples of known related polypeptides are those disclosed in the prior art, such as known orthologs, and paralogs, and similar known members of a protein family, polypeptide or fragments, and the like. For example, a zsig28-specific antibody would not bind to human OSP-like protein, claudin 1, claudin 2 murine CPE receptor or the like. Screening can also be done using non-human zsig28, and zsig28 mutant polypeptides. Moreover, antibodies can be "screened against" known related polypeptides to isolate a population that specifically binds to the inventive polypeptides. For example, antibodies raised to zsig28 are adsorbed to related polypeptides adhered to insoluble matrix; antibodies specific to zsig28 will flow

through the matrix under the proper buffer conditions. Screening allows isolation of polyclonal and monoclonal antibodies non-crossreactive to known closely related polypeptides (Antibodies: A Laboratory Manual, Harlow and Lane (eds.), Cold Spring Harbor Laboratory Press, 1988; Current Protocols in Immunology, Cooligan, et al. (eds.), National Institutes of Health, John Wiley and Sons, Inc., 1995). Screening and isolation of specific antibodies is well known in the art. See, Fundamental Immunology, Paul (eds.), Raven Press, 1993; Getzoff et al., *Adv. in Immunol.* 43: 1-98, 1988; Monoclonal Antibodies: Principles and Practice, Goding, J.W. (eds.), Academic Press Ltd., 1996; Benjamin et al., Ann. Rev. Immunol. 2: 67-101, 1984. Specifically binding anti-zsig28 antibodies can be detected by a number of methods in the art, and disclosed below.

A variety of assays known to those skilled in the art can be utilized to detect antibodies which bind to zsig28 proteins or polypeptides. Exemplary assays are described in detail in Antibodies: A Laboratory Manual, Harlow and Lane (Eds.), Cold Spring Harbor Laboratory Press, 1988. Representative examples of such assays include: concurrent immunoelectrophoresis, radioimmunoassay, radioimmuno-precipitation, enzyme-linked immunosorbent assay (ELISA), dot blot or Western blot assay, inhibition or competition assay, and sandwich assay. In addition, antibodies can be screened for binding to wild-type versus mutant zsig28 protein or polypeptide.

Alternative techniques for generating or selecting antibodies useful herein include *in vitro* exposure of lymphocytes to zsig28 protein or peptide, and selection of antibody display libraries in phage or similar vectors (for instance, through use of immobilized

or labeled zsig28 protein or peptide). Genes encoding polypeptides having potential zsig28 polypeptide binding domains can be obtained by screening random peptide libraries displayed on phage (phage display) or on bacteria, such as *E. coli*. Nucleotide sequences encoding the polypeptides can be obtained in a number of ways, such as through random mutagenesis and random polynucleotide synthesis. These random peptide display libraries can be used to screen for peptides which interact with a known target which can be a protein or polypeptide, such as a ligand or receptor, a biological or synthetic macromolecule, or organic or inorganic substances. Techniques for creating and screening such random peptide display libraries are known in the art (Ladner et al., US Patent NO. 5,223,409; Ladner et al., US Patent NO. 4,946,778; Ladner et al., US Patent NO. 5,403,484 and Ladner et al., US Patent NO. 5,571,698) and random peptide display libraries and kits for screening such libraries are available commercially, for instance from Clontech (Palo Alto, CA), Invitrogen Inc. (San Diego, CA), New England Biolabs, Inc. (Beverly, MA) and Pharmacia LKB Biotechnology Inc. (Piscataway, NJ). Random peptide display libraries can be screened using the zsig28 sequences disclosed herein to identify proteins which bind to zsig28. These "binding polypeptides" which interact with zsig28 polypeptides can be used for tagging cells; for isolating homolog polypeptides by affinity purification; they can be directly or indirectly conjugated to drugs, toxins, radionuclides and the like. These binding polypeptides can also be used in analytical methods such as for screening expression libraries and neutralizing activity, e.g., for blocking interaction between ligand and receptor, or viral binding to a receptor. The binding polypeptides can also be used for

diagnostic assays for determining circulating levels of zsig28 polypeptides; for detecting or quantitating soluble zsig28 polypeptides as marker of underlying pathology or disease. These binding polypeptides can also act as zsig28 "antagonists" to block zsig28 binding and signal transduction *in vitro* and *in vivo*. These anti-zsig28 binding polypeptides would be useful for inhibiting zsig28 activity or protein-binding.

Antibodies to zsig28 may be used for tagging cells that express zsig28; for isolating zsig28 by affinity purification; for diagnostic assays for determining levels of zsig28 polypeptides in normal tissues; for detecting or quantitating zsig28 as marker of underlying pathology or disease; in analytical methods employing FACS; for screening expression libraries; for generating anti-idiotypic antibodies; and as neutralizing antibodies or as antagonists to block zsig28 activity *in vitro* and *in vivo*. Suitable direct tags or labels include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like; indirect tags or labels may feature use of biotin-avidin or other complement/anti-complement pairs as intermediates. Antibodies herein may also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. Moreover, antibodies to zsig28 or fragments thereof may be used *in vitro* to detect denatured zsig28 or fragments thereof in assays, for example, Western Blots or other assays known in the art.

Antibodies or polypeptides herein can also be directly or indirectly conjugated to drugs, toxins, radionuclides and the like, and these conjugates used for *in vivo* diagnostic or therapeutic applications. For instance, polypeptides or antibodies or binding

polypeptides which recognize zsig-28 of the present invention can be used to identify or treat tissues or organs that express a corresponding anti-complementary molecule (e.g, a zsig28 receptor). More specifically, anti-zsig28 antibodies, or bioactive fragments or portions thereof, can be coupled to detectable or cytotoxic molecules and delivered to a mammal having cells, tissues or organs that express the zsig28 molecule.

Suitable detectable molecules may be directly or indirectly attached to polypeptides that bind zsig28 ("binding polypeptides"), antibodies, or bioactive fragments or portions thereof. Suitable detectable molecules include radionuclides, enzymes, substrates, cofactors, inhibitors, fluorescent markers, chemiluminescent markers, magnetic particles and the like. Suitable cytotoxic molecules may be directly or indirectly attached to the polypeptide or antibody, and include bacterial or plant toxins (for instance, diphtheria toxin, *Pseudomonas* exotoxin, ricin, abrin and the like), as well as therapeutic radionuclides, such as iodine-131, rhenium-188 or yttrium-90 (either directly attached to the polypeptide or antibody, or indirectly attached through means of a chelating moiety, for instance). Binding polypeptides or antibodies may also be conjugated to cytotoxic drugs, such as adriamycin. For indirect attachment of a detectable or cytotoxic molecule, the detectable or cytotoxic molecule can be conjugated with a member of a complementary/ anticomplementary pair, where the other member is bound to the binding polypeptide or antibody portion. For these purposes, biotin/streptavidin is an exemplary complementary/ anticomplementary pair.

In another embodiment, binding polypeptide-toxin fusion proteins or antibody-toxin fusion proteins can be used for targeted cell or tissue inhibition or ablation (for instance, to treat cancer cells or tissues). Alternatively, if the binding polypeptide has multiple

functional domains (i.e., an activation domain or a ligand binding domain, plus a targeting domain), a fusion protein including only the targeting domain may be suitable for directing a detectable molecule, a cytotoxic molecule or a complementary molecule to a cell or tissue type of interest. In instances where the domain only fusion protein includes a complementary molecule, the anti-complementary molecule can be conjugated to a detectable or cytotoxic molecule. Such domain-complementary molecule fusion proteins thus represent a generic targeting vehicle for cell/tissue-specific delivery of generic anti-complementary-detectable/ cytotoxic molecule conjugates.

In another embodiment, zsig28 binding polypeptide-cytokine or antibody-cytokine fusion proteins can be used for enhancing *in vivo* killing of target tissues (for example, blood, stomach, colon, and bone marrow cancers), if the binding polypeptide-cytokine or anti-zsig28 antibody targets the hyperproliferative blood or bone marrow cell (See, generally, Hornick et al., Blood 89:4437-47, 1997). For example, Hornick et al. described fusion proteins that enable targeting of a cytokine to a desired site of action, thereby providing an elevated local concentration of cytokine. Suitable anti-zsig28 antibodies target an undesirable cell or tissue (i.e., a tumor or a leukemia), and the fused cytokine mediated improved target cell lysis by effector cells. Suitable cytokines for this purpose include interleukin 2 and granulocyte-macrophage colony-stimulating factor (GM-CSF), for instance.

Alternatively, zsig28 binding polypeptide or antibody fusion proteins described herein can be used for enhancing *in vivo* killing of target tissues by directly stimulating a zsig28-modulated apoptotic pathway, resulting in cell death of hypoproliferative cells expressing zsig28.

The bioactive binding polypeptide or antibody conjugates described herein can be delivered orally, intravenously, intraarterially or intraductally, or may be introduced locally at the intended site of action.

Molecules of the present invention can be used to identify and isolate ligands involved in gastrointestinal function, peristalsis, mucous secretion and the like. For example, proteins and peptides of the present invention can be immobilized on a column and tissue preparations run over the column (Immobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 1992, pp.195-202). Proteins and peptides can also be radiolabeled (Methods in Enzymol., vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-37) or photoaffinity labeled (Brunner et al., Ann. Rev. Biochem. 62:483-514, 1993 and Fedan et al., Biochem. Pharmacol. 33:1167-80, 1984) and specific ligand proteins can be identified.

The polypeptides, nucleic acid and/or antibodies of the present invention can be used in treatment of disorders associated with gastrointestinal cell contractility, secretion of digestive enzymes and acids, gastrointestinal motility, recruitment of digestive enzymes; inflammation, particularly as it affects the gastrointestinal system; reflux disease and regulation of nutrient absorption. Specific conditions that will benefit from treatment with molecules of the present invention include, but are not limited to, diabetic gastroparesis, post-surgical gastroparesis, vagotomy, chronic idiopathic intestinal pseudo-obstruction and gastroesophageal reflux disease. Additional uses include, gastric emptying for radiological studies, stimulating gallbladder contraction and antrectomy.

The motor and neurological affects of molecules of the present invention make it useful for treatment of obesity and other metabolic disorders where neurological feedback modulates nutritional absorption. The molecules of the present invention are useful for regulating satiety, glucose absorption and metabolism, and neuropathy-associated gastrointestinal disorders.

Molecules of the present invention are also useful as additives to anti-hypoglycemic preparations containing glucose and as adsorption enhancers for oral drugs which require fast nutrient action. Additionally, molecules of the present invention can be used to stimulate glucose-induced insulin release.

The polypeptides, nucleic acid, antagonists, agonists and/or antibodies of the present invention can be used in treatment, diagnosis or prevention of disorders associated with gastric ulcers, bacterial diseases, gastric emptying and function, mucosal repair and secretion, stomach cancer, nausea (e.g., induced by cancer therapy and opiate pain control), stomach acid secretion, gastritis, trauma, diverticulitis, gastric mucositis or appetite. The molecules of the present invention can be used to isolate modulators of zsig28, including the natural ligand, used in gene therapy, or to treat or prevent development of pathological conditions in such diverse tissue as stomach and lung.

In addition, zsig28 polypeptides or agonists or antagonists thereof are expected to be useful in the modulation of mucous production, composition or integrity or in a mucous clearing role. Such modulation may be useful in altering mucous composition or integrity for *in vitro* study thereof, such as reducing integrity of mucous to evaluate the implication thereof on bacterial-mucous interaction. In addition, such modulation may be useful in the treatment of disease states characterized by

inappropriate mucous production, composition or integrity. For example, cystic fibrosis is associated with dehydration of the mucous, which results in mucous thickening (reduction in viscosity). Other conditions, such as chronic obstructive pulmonary disease, asthma, and the like, are associated with chronic mucous hypersecretion. See, for example, Prescott et al., Ugeskr Laeger 158(45): 6456-60, 1996; Gordon, Ear Nose Throat J. 75(2): 97-101, 1996; and Jeffery, Am. J. Respir. Crit. Care Med. 150(5 Pt 2): S6-13, 1994. Also, chronic obstructive pulmonary disease and sinonasal inflammatory disease are associated with changes in rhealogical properties or thickening of mucous. See, for example, Agliati, J. Int. Med. Res. 24(3): 302-10, 1996 and Wippold et al., Allergy Proc. 16(4): 165-9, 1995. In addition, mucous structural integrity is adversely impacted in inflammatory bowel disease, possibly via increased proteolysis. See, for example, Playford et al., Amer. J. Pathol. 146(2): 310-6, 1995. Certain forms of chronic obstructive pulmonary disease are associated with increased acidic mucous. See, for example, Jeffery, supra.. Mucous clearing may be useful in a number of these conditions as well.

The zsig28 polypeptide is expressed in the stomach. As a receptor, zsig28 can play important roles in the maintenance of normal gastric epithelium and function. Thus, zsig28 polypeptide pharmaceutical compositions, agonists and antagonists of the present invention may also be useful in prevention or treatment of gastric mucositis. Mucositis is manifested by the damage and loss of integrity of the oral and gastric epithelium. Such damage often provides a microbial port of entry leading to sepsis. Mucositis is often induced by chemotherapy and radiation therapy, and is often a dose-limiting side effect as well as a cause of mortality in

cancer patients undergoing such treatment. The zsig28 polypeptides and agonists and antagonists of the present invention may provide protection against gastric mucositis, analogous to some growth factors and cytokines, for example, interleukin-11 (Orazi, A. et al., Lab. Invest. 75:33-42, 1996). The effect of zsig28, agonists and antagonists in prevention or treatment of gastric mucositis can be measured in *in vivo* animal models, for example, the Syrian hamster model or in murine models using methods described in the art (Sonis, S.T. et al., Oral Surg. Oral Med. Oral Pathol. 69:437-443, 1990; Farrell, C.L. et al., Cancer Res. 58:933-939, 1998; Orazi, A. et al., supra.). Moreover, zsig28 transgenic or knockout mice may provide an additional *in vivo* model for gastric mucositis.

To verify these capabilities in zsig28 polypeptides of the present invention, agonists, or antagonists, the zsig28 polypeptides, agonists or antagonists are evaluated for mucosal integrity maintenance activity according to procedures known in the art. See, for example, Zahm et al., Eur. Respir. J. 8: 381-6, 1995, which describes methods for measuring viscoelastic properties and surface properties of mucous as well as for evaluating mucous transport by cough and by ciliary activity. Other assays for evaluating the properties of mucous are known to those of ordinary skill in the art. Such assays include those for determining mucin content, water content, carbohydrate content, intrinsic buffering capacity, acidity, barrier properties, ability to absorb water and the like.

Moreover, detection of zsig28 polypeptides in the serum, mucous or tissue biopsy of a patient undergoing evaluation for or disorders characterized by inappropriate mucous deposition, composition or properties, such as cystic fibrosis, asthma, bronchitis, inflammatory bowel

disease, Crohn's disease, chronic obstructive pulmonary disease or the like, can be employed in a diagnostic application of the present invention. Such zsig28 polypeptides can be detected using immunoassay techniques and antibodies capable of recognizing a zsig28 polypeptide epitope. As an illustration, the present invention contemplates methods for detecting zsig28 polypeptide comprising:

exposing a sample possibly containing zsig28 polypeptide to an antibody attached to a solid support, wherein said antibody binds to an epitope of a zsig28 polypeptide;

washing said immobilized antibody-polypeptide to remove unbound contaminants;

exposing the immobilized antibody-polypeptide to a second antibody directed to a second epitope of a zsig28 polypeptide, wherein the second antibody is associated with a detectable label; and

detecting the detectable label. An increase or decrease in the concentrations of zsig28 polypeptide (in comparison to normal concentrations thereof) in the test sample appears to be indicative of dysfunction. One of skill in the art would appreciate that other assays known in the art can be used to detect zsig28 in a test sample, for example, a simple solution assay with labeled anti-zsig28 antibody.

In addition, pharmaceutical compositions containing such mucosa-modulating agents may be employed in the treatment of disorders associated with alterations in mucosal production, composition or integrity, such as those described above. Such patients will be given an effective amount of zsig28 polypeptide or agonist or antagonist thereof having mucosal-modulating activity to achieve a therapeutic benefit, generally manifested in a

change in mucosal production, composition or integrity in the direction of the normal physiological state thereof.

Also, the zsig28 polypeptides of the present invention are found in high abundance in digestive tissues, such as stomach. Thus, expression of zsig28 polypeptides may serve as a marker for digestive function or to promote digestive organ proliferation or differentiation. Also, zsig28 polypeptides or agonists or antagonists thereof may be useful in modulating the lubrication or barrier properties of digestive organ mucosa.

Zsig28 polypeptides of the present invention or agonists or antagonists thereof may be used as anti-microbial agents to protect against pathological action of microorganisms. Such anti-bacterial agents are preferably active on mucosa-associated microorganisms, such as *C. albicans, pneumonus, hemophilus, Helicobacter pylori,* and the like. An example of a microbial-associated condition with mucous involvement in humans is the diminution of the defensive properties of the gastroduodenal mucosa by *H. pylori,* potentially resulting in ulcer formation. See, for example, Beligotskii et al., Klin. Khir. 8: 3-6, 1994.

These anti-microbial protective agents may be directly acting or indirectly acting. Such agents operating via membrane association or pore forming mechanisms of action directly attach to the offending microbe. Anti-microbial agents can also act via an enzymatic mechanism, breaking down microbial protective substances or the cell wall/membrane thereof. Anti-microbial agents, capable of inhibiting microorganism proliferation or action or of disrupting microorganism integrity by either mechanism set forth above, are useful in methods for preventing contamination in cell culture by microbes susceptible to that anti-microbial activity.

Such techniques involve culturing cells in the presence of an effective amount of said zsig28 secreted region 1, 2, 3, or 4 polypeptide fragment, or a zsig28 agonist or antagonist. Assays to evaluate the efficacy of zsig28 polypeptides, agonists or antagonists thereof as anti-microbial agents are known in the art.

Moreover, detection of zsig28 polypeptides in the serum, mucous or tissue biopsy of a patient undergoing evaluation for microbial disorders, particularly those associated with mucosa, can be employed in a diagnostic application of the present invention. Such zsig28 polypeptides can be detected using immunoassay techniques and antibodies capable of recognizing a zsig28 polypeptide epitope, as described herein. Altered levels of zsig28 polypeptides in a test sample, such as serum sweat, saliva, biopsy, and the like, can be monitored as an indication of digestive function, gastric ulcer or of cancer or disease, when compared against a normal control.

In addition, pharmaceutical compositions containing such anti-microbial agents may be employed in the treatment of microbial disorders, particularly those associated with mucosa. Such patients will be given an effective amount of zsig28 soluble polypeptide fragment or agonist or antagonist thereof having anti-microbial activity to achieve a therapeutic benefit, generally manifested in a decrease in proliferation or function of the pathogenic microbe. Other conditions which may be addressed in accordance with the present invention are eye, nasal, oral and rectal conditions involving the mucosa and/or pathological microbial agents, chemotherapy side effects impacting the mucosa, AIDS complications relating to mucosa or the like. The anti-microbial activity of zsig28 soluble polypeptide fragment, agonists or antagonists may be determined using known assays therefore. See, for example, Barsum et al., Eur. Respir.

J. 8(5): 709-14, 1995; Sandovsky-Losica et al., J. Med. Vet. Mycol (England) 28(4): 279-87, 1990; Mehentee et al., J. Gen. Microbiol (England) 135 (Pt. 8): 2181-8, 1989; Segal and Savage, Journal of Medical and Veterinary Mycology 24: 477-479, 1986, and the like.

Also, zsig28 polypeptides of the present invention may constitute a component of a known tissue glue, imparting additional adhesive and/or anti-microbial properties thereto. In such applications, purified zsig28 polypeptide would be used in combination with collagen or a form of gelatin, muscle adhesion protein, fibrinogen, thrombin, Factor XIII or the like. The different types of tissue glues as well as the composition thereof are known in the art.

Zsig28 can also be used to identify modulators (e.g, antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of zsig28. In addition to those assays disclosed herein, samples can be tested for inhibition of zsig28 activity within a variety of assays designed to measure zsig28 binding, oligomerization, or the stimulation/inhibition of zsig28-dependent cellular responses. For example, zsig28-expressing cell lines can be transfected with a reporter gene construct that is responsive to a zsig28-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a zsig28-DNA response element operably linked to a gene encoding an assay detectable protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., Proc. Natl. Acad. Sci. USA 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. Cell 56: 563-72, 1989).

Cyclic AMP response elements are reviewed in Roestler et al., J. Biol. Chem. 263 (19):9063-6; 1988 and Habener, Molec. Endocrinol. 4 (8):1087-94; 1990. Hormone response elements are reviewed in Beato, Cell 56:335-44; 1989. Candidate compounds, solutions, mixtures or extracts are tested for the ability to inhibit the activity of zsig28 on the target cells as evidenced by a decrease in zsig28 stimulation of reporter gene expression. Assays of this type will detect compounds that directly block zsig28 binding to cell-surface receptors, e.g., through dimerization, as well as compounds that block processes in the cellular pathway subsequent to such binding. As such, there is provided a method of identifying antagonists of zsig28 polypeptide, comprising providing cells responsive to a zsig28 polypeptide, culturing a first portion of the cells in the presence of zsig28 polypeptide, culturing a second portion of the cells in the presence of the zsig28 polypeptide and a test compound, and detecting a decrease in a cellular response of the second portion of the cells as compared to the first portion of the cells. Moreover, compounds or other samples can be tested for direct blocking of zsig28, or blocking of zsig28 binding to other cell surface molecules, using zsig28 tagged with a detectable label (e.g., ^{125}I, biotin, horseradish peroxidase, FITC, and the like). Within assays of this type, the ability of a test sample to inhibit labeled zsig28 binding to another protein can be indicative of inhibitory activity, which can be confirmed through secondary assays. Antagonists are therefore useful to inhibit or diminish zsig28 polypeptide function. Alternatively, there is provided a method of identifying zsig28 polypeptide agonists, comprising providing cells expressing a zsig28 polypeptide as disclosed above, culturing the cells in the presence of a test compound and

comparing the cellular response with the cell cultured in the presence of the zsig28 polypeptide, and selecting the test compounds for which the cellular response is of the same type. Agonists are therefore useful to mimic a zsig28 ligand and/or to augment the function of zsig28 polypeptides.

In view of the tissue distribution observed for zsig28, agonists (including the natural ligand/ substrate/ cofactor/ etc.) and antagonists have enormous potential in both *in vitro* and *in vivo* applications. Compounds identified as zsig28 agonists are useful for promoting apoptosis in cells over-expressing sig58, *in vitro* and *in vivo*, such as tumor cells. Compounds identified as zsig28 agonists are also useful for and stimulating cell growth or differentiation, of various cell types. For example, zsig28 agonist compounds are useful as components of defined cell culture media, and may be used alone or in combination with other cytokines and hormones to replace serum that is commonly used in cell culture. Also, zsig28 polypeptide can be hydrolyzed to provide a source of amino acids to cultured cells. Moreover, the zsig28 polypeptides and zsig28 agonist polypeptides are useful as a research reagent, such as for the expansion of stomach-derived, or intestinal cells. Zsig28 agonists can be added to tissue culture media for cell types expressing zsig28 polypeptide.

Inhibitors of zsig28 activity (zsig28 antagonists) include anti-zsig28 antibodies and polypeptide binding fragments, as well as other peptidic and non-peptidic agents (including ribozymes). Zsig28 can also be used to identify inhibitors (antagonists) of its activity. Test compounds are added to the assays disclosed herein to identify compounds that inhibit the activity of zsig28. In addition to those assays disclosed

herein, samples can be tested for inhibition of zsig28 activity within a variety of assays designed to measure receptor binding or the stimulation/inhibition of zsig28-dependent cellular responses. For example, zsig28-responsive cell lines can be transfected with a reporter gene construct that is responsive to a zsig28-stimulated cellular pathway. Reporter gene constructs of this type are known in the art, and will generally comprise a zsig28-DNA response element operably linked to a gene encoding an assayable protein, such as luciferase. DNA response elements can include, but are not limited to, cyclic AMP response elements (CRE), hormone response elements (HRE) insulin response element (IRE) (Nasrin et al., Proc. Natl. Acad. Sci. USA 87:5273-7, 1990) and serum response elements (SRE) (Shaw et al. Cell 56: 563-72, 1989). Cyclic AMP response elements are reviewed in Roestler et al., J. Biol. Chem. 263 (19):9063-6; 1988 and Habener, Molec. Endocrinol. 4 (8):1087-94; 1990. Hormone response elements are reviewed in Beato, Cell 56:335-44; 1989. Candidate compounds, solutions, mixtures or extracts are tested for the ability to inhibit the activity of zsig28 polypeptide on the target cells as evidenced by a decrease in zsig28 stimulation of reporter gene expression. Assays of this type will detect compounds that directly block zsig28 ligands from binding to zsig28 polypeptide receptors, or receptor multimerization, as well as compounds that block processes in the cellular pathway subsequent to receptor-ligand binding.

Molecules of the present invention can be used to identify and isolate zsig28 receptors involved or present in cancer metastases. Thus, the zsig28 polypeptide can serve as a diagnostic for cancer metastasis. For example, proteins and peptides of the

present invention can be immobilized on a column and membrane preparations run over the column (Immobilized Affinity Ligand Techniques, Hermanson et al., eds., Academic Press, San Diego, CA, 1992, pp.195-202). (Methods in Enzymol., vol. 182, "Guide to Protein Purification", M. Deutscher, ed., Acad. Press, San Diego, 1990, 721-737) or photoaffinity labeled (Brunner et al., Ann. Rev. Biochem. 62:483-514, 1993 and Fedan et al., Biochem. Pharmacol. 33:1167-1180, 1984) and zsig28 cell-surface proteins can be identified. Moreover, using methods known in the art, antibodies to zsig28 can also be radiolabeled, fluorescent or chemically labeled and used in histological assays to detect elevated zsig28 present in biopsies. Polypeptides of the present invention are useful for measuring changes in levels of expression of zsig28 polypeptides. Because zsig28 expression is restricted to specific tissues (i.e., stomach and lung), changes in expression levels could be used to monitor metabolism within these tissues. For example, increases in expression and/or transcription of zsig28 polypeptides and polynucleotides, may be predictive for increased cell proliferation of tumor cells. Furthermore, expression of zsig28 in tissue not normally expressing zsig28, may be indicative of metastasis of tumor cells.

Zsig28 may be demonstrated to be expressed differentially in certain epithelial tissues and carcinomas, particularly in stomach, colon, esophagus, or intestine. Differential expression is the transient expression, or lack thereof, of specific genes, proteins or other phenotypic properties (known as differentiation markers) that occur during the progress of maturation in a cell or tissue. A set of differentiation markers is defined as one or more phenotypic properties that can be identified and are specific to a particular cell type.

Thus, pluripotent stem cells that can regenerate without commitment to a lineage express a set of differentiation markers that are lost when commitment to a cell lineage is made. Precursor cells express a set of differentiation markers that may or may not continue to be expressed as the cells progress down the cell lineage pathway toward maturation. Differentiation markers that are expressed exclusively by mature cells are usually functional properties such as cell products, enzymes to produce cell products and receptors.

Zsig28 expression can be used as a differentiation marker in normal and tumor tissues to determine the stage of the tumor or maturity of a cell. Zsig28 will be particularly valuable as a marker for epithelial cells and tumor of epithelial origin, and more particularly epithelial cells and epithelial-derived tumors from stomach tissues.

A set of differentiation markers is defined as one or more phenotypic properties that can be identified and are specific to a particular cell type. Differentiation markers are transiently exhibited at various stages of cell lineage. Pluripotent stem cells that can regenerate without commitment to a lineage express a set of differentiation markers that are lost when commitment to a cell lineage is made. Precursor cells express a set of differentiation markers that may or may not continue to be expressed as the cells progress down the cell lineage pathway toward maturation. Differentiation markers that are expressed exclusively by mature cells are usually functional properties such as cell products, enzymes to produce cell products and receptors. The activity of molecules of the present invention can be measured using a variety of assays that measure proliferation and/or differentiation of specific

cell types, chemotaxis, adhesion, changes in ion channel influx, regulation of second messenger levels and neurotransmitter release. Such assays are well known in the art and described herein.

Additional methods using probes or primers derived, for example, from the nucleotide sequences disclosed herein can also be used to detect zsig28 expression in a patient sample, such as a tumor biopsy, stomach, lung, blood, saliva, tissue sample, or the like. For example, probes can be hybridized to tumor tissues and the hybridized complex detected by *in situ* hybridization. Zsig28 sequences can also be detected by PCR amplification using cDNA generated by reverse translation of sample mRNA as a template (PCR Primer A Laboratory Manual, Dieffenbach and Dveksler, eds., Cold Spring Harbor Press, 1995). When compared with a normal control, both increases or decreases of zsig28 expression in a patient sample, relative to that of a control, can be monitored and used as an indicator or diagnostic for disease.

Polynucleotides encoding zsig28 polypeptides are useful within gene therapy applications where it is desired to increase or inhibit zsig28 activity. If a mammal has a mutated or absent zsig28 gene, the zsig28 gene can be introduced into the cells of the mammal. Moreover, using gene therapy applications zsig28 can also be used directly as a chemotherapeutic agent. For example, using methods disclosed herein, zsig28 can be directly introduced into cancer cells to trigger apoptosis and cell death. In one embodiment, a gene encoding a zsig28 polypeptide is introduced *in vivo* in a viral vector. Such vectors include an attenuated or defective DNA virus, such as, but not limited to, herpes simplex virus (HSV), papillomavirus, Epstein Barr virus (EBV), adenovirus, adeno-associated virus (AAV), and the like. Defective viruses, which entirely or almost entirely lack viral genes, are preferred. A defective virus is not

infective after introduction into a cell. Use of defective viral vectors allows for administration to cells in a specific, localized area, without concern that the vector can infect other cells. Examples of particular vectors include, but are not limited to, a defective herpes simplex virus 1 (HSV1) vector (Kaplitt et al., Molec. Cell. Neurosci. 2:320-30, 1991); an attenuated adenovirus vector, such as the vector described by Stratford-Perricaudet et al., J. Clin. Invest. 90:626-30, 1992; and a defective adeno-associated virus vector (Samulski et al., J. Virol. 61:3096-101, 1987; Samulski et al., J. Virol. 63:3822-8, 1989).

In another embodiment, a zsig28 gene can be introduced in a retroviral vector, e.g., as described in Anderson et al., U.S. Patent No. 5,399,346; Mann et al. Cell 33:153, 1983; Temin et al., U.S. Patent No. 4,650,764; Temin et al., U.S. Patent No. 4,980,289; Markowitz et al., J. Virol. 62:1120, 1988; Temin et al., U.S. Patent No. 5,124,263; International Patent Publication No. WO 95/07358, published March 16, 1995 by Dougherty et al.; and Kuo et al., Blood 82:845, 1993. Alternatively, the vector can be introduced by lipofection *in vivo* using liposomes. Synthetic cationic lipids can be used to prepare liposomes for *in vivo* transfection of a gene encoding a marker (Felgner et al., Proc. Natl. Acad. Sci. USA 84:7413-7, 1987; Mackey et al., Proc. Natl. Acad. Sci. USA 85:8027-31, 1988). The use of lipofection to introduce exogenous genes into specific organs *in vivo* has certain practical advantages. Molecular targeting of liposomes to specific cells represents one area of benefit. More particularly, directing transfection to particular cells represents one area of benefit. For instance, directing transfection to particular cell types would be particularly advantageous in a tissue with cellular heterogeneity, such as the pancreas, liver, kidney, and brain. Lipids may be chemically coupled to

other molecules for the purpose of targeting. Targeted peptides (e.g., hormones or neurotransmitters), proteins such as antibodies, or non-peptide molecules can be coupled to liposomes chemically.

It is possible to remove the target cells from the body; to introduce the vector as a naked DNA plasmid; and then to re-implant the transformed cells into the body. Naked DNA vectors for gene therapy can be introduced into the desired host cells by methods known in the art, e.g., transfection, electroporation, microinjection, transduction, cell fusion, DEAE dextran, calcium phosphate precipitation, use of a gene gun or use of a DNA vector transporter. See, e.g., Wu et al., J. Biol. Chem. 267:963-7, 1992; Wu et al., J. Biol. Chem. 263:14621-4, 1988.

Antisense methodology can be used to inhibit zsig28 gene transcription, such as to inhibit cell proliferation in vivo. Polynucleotides that are complementary to a segment of a zsig28-encoding polynucleotide (e.g., a polynucleotide as set froth in SEQ ID NO:1) are designed to bind to zsig28-encoding mRNA and to inhibit translation of such mRNA. Such antisense polynucleotides are used to inhibit expression of zsig28 polypeptide-encoding genes in cell culture or in a subject.

In addition, as a cell surface molecule, zsig28 polypeptide can be used as a target to introduce gene therapy into a cell. This application would be particularly appropriate for introducing therapeutic genes into cells in which zsig28 is normally expressed, such as stomach tissue and lung, or cancer cells which express zsig28 polypeptide. For example, viral gene therapy, such as described above, can be targeted to specific cell types in which express a cellular receptor, such as zsig28 polypeptide, rather than the viral receptor. Antibodies, or other molecules that recognize zsig28 molecules on the

target cell's surface can be used to direct the virus to infect and administer gene therapeutic material to that target cell. See, Woo, S.L.C, Nature Biotech. 14:1538, 1996; Wickham, T.J. et al, Nature Biotech. 14:1570-1573, 1996; Douglas, J.T et al., Nature Biotech. 14:1574-1578, 1996; Rihova, B., Crit. Rev. Biotechnol. 17:149-169, 1997; and Vile, R.G. et al., Mol. Med. Today 4:84-92, 1998. For example, a bispecific antibody containing a virus-neutralizing Fab fragment coupled to a zsig28-specific antibody can be used to direct the virus to cells expressing the zsig28 receptor and allow efficient entry of the virus containing a genetic element into the cells. See, for example, Wickham, T.J., et al., J. Virol. 71:7663-7669, 1997; and Wickham, T.J., et al., J. Virol. 70:6831-6838, 1996.

The present invention also provides reagents which will find use in diagnostic applications. For example, the zsig28 gene, a probe comprising zsig28 DNA or RNA or a subsequence thereof can be used to determine if the zsig28 gene is present on chromosome 3 or if a mutation has occurred. Zsig28 is located at the 3q22.1-3q22.2 region of chromosome 3 (See, Example 3). Detectable chromosomal aberrations at the zsig28 gene locus include, but are not limited to, aneuploidy, gene copy number changes, insertions, deletions, restriction site changes and rearrangements. Such aberrations can be detected using polynucleotides of the present invention by employing molecular genetic techniques, such as restriction fragment length polymorphism (RFLP) analysis, short tandem repeat (STR) analysis employing PCR techniques, and other genetic linkage analysis techniques known in the art (Sambrook et al., ibid.; Ausubel et. al., ibid.; Marian, Chest 108:255-65, 1995).

The precise knowledge of a gene's position can be useful for a number of purposes, including: 1)

determining if a sequence is part of an existing contig and obtaining additional surrounding genetic sequences in various forms, such as YACs, BACs or cDNA clones; 2) providing a possible candidate gene for an inheritable disease which shows linkage to the same chromosomal region; and 3) cross-referencing model organisms, such as mouse, which may aid in determining what function a particular gene might have.

The zsig28 gene is located at the 3q22.1-3q22.2 region of chromosome 3. Several genes of known function map to this region. For example, angiotensin receptor 1 is mapped to 3q21-q25 and is related to hypertension in humans since it controls blood pressure. In addition, zsig28 polynucleotide probes can be used to detect abnormalities or genotypes associated with tumor associated antigen L6, which maps to 3q21-q25, and is highly expressed in lung breast and colon cancers (Marken et al, Proc. Nat. Acad. Sci. 89:3503-3507, 1992). Moreover, amongst other genetic loci, those for alkaptoneuria (3q21-q23), Moebius 2 syndrome (3q21-q22), open angle glaucoma (3q21-q24), calcium sensing receptor (3q21-q24) all manifest themselves in human disease states as well as map to this region of the human genome. See the Online Mendellian Inheritance of Man (OMIM) gene map, and references therein, for this region of chromosome 3 on a publicly available WWW server (http://www3.ncbi.nlm.nih.gov/htbin-post/Omim/getmap?chromosome=3q22.1). All of these serve as possible candidate genes for an inheritable disease which show linkage to the same chromosomal region as the zsig28 gene.

Moreover, a gene related to zsig28, the human transmembrane protein deleted in velo-cardio-facial syndrome (TMVCF) (Sirotkin H. et al., Genomics 42:245-251, 1997) is a marker for a human autosomal dominant disorder velo-cardio-facial syndrome (VCFS) characterized amongst

other symptoms by facial deformation, mental retardation, and heart defects and is deleted in 80-85% of patients with this syndrome. Moreover, mutations in another zsig28-related gene, peripheral myelin protein-22 (PMP-22), are present in the human Charcot-Marie-Tooth neuropathy (CMT) with a phenotypically similar neuropathy in a PMP-22 transgenic murine mouse model (Erdem, S. et al., J Neuropathol. Exp. Neurol. 57:635-642, 1998; Fabbretti, E. et al., Genes Dev. 15:1846-1856, 1995; Magyar, J.P. et al., J Neurosci. 16:5351-5360, 1996; Adlkofer, K. et al., Nat. Genet. 11:274-280, 1995). Similarly, defects in the zsig28 locus itself may result in a heritable human disease state. Molecules of the present invention, such as the polypeptides, antagonists, agonists, polynucleotides and antibodies of the present invention would aid in the detection, diagnosis prevention, and treatment associated with a zsig28 genetic defect.

Mice engineered to express the zsig28 gene, referred to as "transgenic mice," and mice that exhibit a complete absence of zsig28 gene function, referred to as "knockout mice," may also be generated (Snouwaert et al., Science 257:1083, 1992; Lowell et al., Nature 366:740-42, 1993; Capecchi, M.R., Science 244: 1288-1292, 1989; Palmiter, R.D. et al. Annu Rev Genet. 20: 465-499, 1986). For example, transgenic mice that over-express zsig28, either ubiquitously or under a tissue-specific or tissue-restricted promoter can be used to ask whether over-expression causes a phenotype. For example, over-expression of a wild-type zsig28 polypeptide, polypeptide fragment or a mutant thereof may alter normal cellular processes, resulting in a phenotype that identifies a tissue in which zsig28 expression is functionally relevant and may indicate a therapeutic target for the zsig28,its agonists or antagonists. For example, a preferred transgenic mouse to engineer is one that over-expresses

the zsig28 mature polypeptide (approximately amino acids 24 (Ala) to 261 (Val) of SEQ ID NO:2). Moreover, such over-expression may result in a phenotype that shows similarity with human diseases. Similarly, knockout zsig28 mice can be used to determine where zsig28 is absolutely required *in vivo*. The phenotype of knockout mice is predictive of the *in vivo* effects of that a zsig28 antagonist, such as those described herein, may have. The human zsig28 cDNA can be used to isolate murine zsig28 mRNA, cDNA and genomic DNA, which are subsequently used to generate knockout mice. These mice may be employed to study the zsig28 gene and the protein encoded thereby in an *in vivo* system, and can be used as *in vivo* models for corresponding human diseases. Moreover, transgenic mice expression of zsig28 antisense polynucleotides or ribozymes directed against zsig28, described herein, can be used analogously to transgenic mice described above.

The invention is further illustrated by the following non-limiting examples.

EXAMPLES

Example 1

Identification of zsig28

A. Using an EST Sequence to Obtain Full-length zsig28

Scanning of a translated lung library DNA database using a signal trap as a query resulted in identification of an expressed sequence tag (EST) sequence found to be homologous to a human secretory signal sequence.

Confirmation of the EST sequence was made by sequence analyses of the cDNA from which the EST originated. This cDNA was contained in a plasmid, and was sequenced using the following primers: ZC447(SEQ ID

NO:11), ZC12501 (SEQ ID NO:12), ZC 12502 (SEQ ID NO:13), and ZC 976 (SEQ ID NO:14). The clone appeared to be full length.

Example 2

Tissue Distribution

Northern blot analysis was performed using Human Multiple Tissue Northern™ Blots (MTN I, MTN II, and MTN III) (Clontech). An insert from the full length clone, described in Example 1, was excised using EcoRI and NotI (Boehringer) and gel purified using a commercially available kit (QiaexII™; Qiagen) and then radioactively labeled with ^{32}P-dCTP using Rediprime™ (Amersham), a random prime labeling system, according to the manufacturer's specifications. The probe was then purified using a Nuc-Trap™ column (Stratagene) according to the manufacturer's instructions. ExpressHyb™ (Clontech) solution was used for prehybridization and as a hybridizing solution for the Northern blots. Hybridization took place overnight at 42°C using 3 x 10^6 cpm/ml of labeled probe. The blots were then washed in 2X SSC/1% SDS at room temperature, followed by a wash in 0.1X SSC/0.1% SDS at 65°C. An approximately 4 kb transcript was strongly detected in stomach and weakly detected in lung. No signals were apparent in other tissues represented on the blots.

Dot Blots were also performed using Human RNA Master Blots™ (Clontech). The methods and conditions for the Dot Blots are the same as for the Multiple Tissue Blots described above. Strong signal intensity was present in stomach, with detectable but low expression in adult and fetal lung.

Example 3

PCR-Based Chromosomal Mapping of the zsig28 Gene

Zsig28 was mapped to chromosome 3 using the commercially available "GeneBridge 4 Radiation Hybrid Panel" (Research Genetics, Inc., Huntsville, AL). The GeneBridge 4 Radiation Hybrid Panel contains DNAs from each of 93 radiation hybrid clones, plus two control DNAs (the HFL donor and the A23 recipient). A publicly available WWW server (http://www-genome.wi.mit.edu/cgi-bin/contig/rhmapper.pl) allows mapping relative to the Whitehead Institute/MIT Center for Genome Research's radiation hybrid map of the human genome (the "WICGR" radiation hybrid map) which was constructed with the GeneBridge 4 Radiation Hybrid Panel.

For the mapping of Zsig28 with the "GeneBridge 4 RH Panel", 20 µl reactions were set up in a 96-well microtiter plate (Stratagene, La Jolla, CA) and used in a "RoboCycler Gradient 96" thermal cycler (Stratagene). Each of the 95 PCR reactions consisted of 2 µl 10X KlenTaq PCR reaction buffer (Clontech Laboratories, Inc., Palo Alto, CA), 1.6 µl dNTPs mix (2.5 mM each, PERKIN-ELMER, Foster City, CA), 1 µl sense primer, ZC19,410 (SEQ ID NO:15), 1 µl antisense primer, ZC19,411 (SEQ ID NO:16), 2 µl "RediLoad" (Research Genetics), 0.4 µl 50X Advantage KlenTaq™ Polymerase Mix (Clontech), 25 ng of DNA from an individual hybrid clone or control and ddH_2O for a total volume of 20 µl. The reactions were overlaid with an equal amount of mineral oil and sealed. The PCR cycler conditions were as follows: an initial 1 cycle 5 minute denaturation at 95°C, 35 cycles of a 1 minute denaturation at 95°C, 1 minute annealing at 60°C and 1.5 minute extension at 72°C, followed by a final 1 cycle extension of 7 minutes at 72°C. The reactions were separated by

electrophoresis on a 2% agarose gel (Life Technologies, Gaithersburg, MD).

The results showed that Zsig28 maps 4.40 cR_3000 from the framework marker D3S1576 on the WICGR chromosome 3 radiation hybrid map. Proximal and distal framework markers were D3S1576 and WI-3522, respectively. The use of surrounding markers positions Zsig28 in the 3q22.1-3q22.2 region on the integrated LDB chromosome 3 map (The Genetic Location Database, University of Southhampton, WWW server: http://cedar.genetics. soton.ac.uk/public_html/).

From the foregoing, it will be appreciated that, although specific embodiments of the invention have been described herein for purposes of illustration, various modifications may be made without deviating from the spirit and scope of the invention. Accordingly, the invention is not limited except as by the appended claims.

CLAIMS

What is claimed is:

1. An isolated polynucleotide that encodes a polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val),

wherein the amino acid percent identity is determined using a FASTA program with ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62, with other parameters set as default.

2. An isolated polynucleotide according to claim 1, wherein the polynucleotide is selected from the group consisting of:

(a) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 139 to nucleotide 853; and

(b) a polynucleotide sequence as shown in SEQ ID NO:1 from nucleotide 70 to nucleotide 853.

3. An isolated polynucleotide sequence according to claim 1, wherein the polynucleotide comprises nucleotide 1 to nucleotide 783 of SEQ ID NO:10.

4. An isolated polynucleotide according to claim 1, wherein the zsig28 polypeptide comprising a sequence of amino acid residues selected from the group consisting of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val).

5. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA segment encoding a zsig28 polypeptide as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

a transcription terminator,

wherein the promoter is operably linked to the DNA segment, and the DNA segment is operably linked to the transcription terminator.

6. An expression vector according to claim 5, further comprising a secretory signal sequence operably linked to the DNA segment.

7. A cultured cell comprising an expression vector according to claim 5, wherein the cell expresses a polypeptide encoded by the DNA segment.

8. A DNA construct encoding a fusion protein, the DNA construct comprising:

a first DNA segment encoding a polypeptide comprising a sequence of amino acid residues selected from the group consisting of:

(a) the amino acid sequence of SEQ ID NO:2 from amino acid number 1 (Met), to amino acid number 23 (Ala);

(b) the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala) to amino acid number 82 (Leu);

(c) the amino acid sequence of SEQ ID NO:2 from amino acid number 101 (Leu) to amino acid number 122 (Gly);

(d) the amino acid sequence of SEQ ID NO:2 from amino acid number 141 (Asn) to amino acid number 174 (Ala);

(e) the amino acid sequence of SEQ ID NO:2 from amino acid number 193 (Cys) to amino acid number 261 (Val); and

(f) the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

at least one other DNA segment encoding an additional polypeptide,

wherein the first and other DNA segments are connected in-frame; and

wherein the first and other DNA segments encode the fusion protein.

9. An expression vector comprising the following operably linked elements:

a transcription promoter;

a DNA construct encoding a fusion protein according to claim 8; and

a transcription terminator,

wherein the promoter is operably linked to the DNA construct, and the DNA construct is operably linked to the transcription terminator.

10. A cultured cell comprising an expression vector according to claim 9, wherein the cell expresses a polypeptide encoded by the DNA construct.

11. A method of producing a fusion protein comprising:

culturing a cell according to claim 10; and

isolating the polypeptide produced by the cell.

12. An isolated polypeptide comprising a sequence of amino acid residues that is at least 90% identical to an amino acid sequence selected from the group consisting of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val),

wherein the amino acid percent identity is determined using a FASTA program with ktup=1, gap opening penalty=10, gap extension penalty=1, and substitution matrix=BLOSUM62, with other parameters set as default.

13. An isolated polypeptide according to claim 12, wherein the polypeptide further contains motifs 1 through 4 spaced apart from N-terminus to C-terminus in a configuration selected from the group consisting of:

(a) Met-{47-50}-M1-{21-22}-M2-{73-92}-M3; and

(b) Met-{47-50}-M1-{21-22}-M2-{73-92}-M3-{3}-M4,

wherein M1 is "motif 1," a sequence of amino acids as shown in amino acids 48 to 54 of SEQ ID NO:2,

M2 is "motif 2," a sequence of amino acids as shown in amino acids 77 to 82 of SEQ ID NO:2,

M3 is "motif 3," a sequence of amino acids as shown in amino acids 174 to 180 of SEQ ID NO:2,

M4 is "motif 4," a sequence of amino acids as shown in amino acids 184 to 189 of SEQ ID NO:2, and

{#} denotes the number of amino acids between the motifs.

14. An isolated polypeptide according to claim 12, wherein the polypeptide comprises a sequence of amino acid residues selected from the group consisting of:

(a) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val); and

(b) the amino acid sequence as shown in SEQ ID NO:2 from amino acid number 1 (Met) to amino acid number 261 (Val).

15. A method of producing a zsig28 polypeptide comprising:

culturing a cell according to claim 7; and

isolating the zsig28 polypeptide produced by the cell.

16. A method of producing an antibody to zsig28 polypeptide comprising:

inoculating an animal with a polypeptide selected from the group consisting of:

(a) a polypeptide consisting of 9 to 238 amino acids, wherein the polypeptide is identical to a contiguous sequence of amino acids in SEQ ID NO:2 from amino acid number 24 (Ala), to amino acid number 261 (Val);

(b) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 24 (Ala) to amino acid number 82 (Leu);

(c) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 101 (Leu) to amino acid number 122 (Gly);

(d) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 141 (Asn) to amino acid number 174 (Ala);

(e) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 193 (Cys) to amino acid number 261 (Val);

(f) a polypeptide according to claim 12;

(g) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 245 (Ala) to amino acid number 250 (Glu);

(h) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 234 (Asn) to amino acid number 239 (Lys);

(i) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 202 (Glu) to amino acid number 207 (Lys);

(j) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 254 (Lys) to amino acid number 259 (Asp); and

(k) a polypeptide consisting of the amino acid sequence of SEQ ID NO:2 from amino acid number 110 (Glu) to amino acid number 115 (Ala); and

wherein the polypeptide elicits an immune response in the animal to produce the antibody; and

isolating the antibody from the animal.

17. An antibody produced by the method of claim 16, which binds to a zsig28 polypeptide.

18. The antibody of claim 17, wherein the antibody is a monoclonal antibody.

19. An antibody which specifically binds to a polypeptide of claim 12.

20. A method of detecting, in a test sample, the presence of a modulator of zsig28 protein activity, comprising:

culturing a cell into which has been introduced an expression vector according to claim 5, wherein the cell expresses the zsig28 protein encoded by the DNA segment in the presence and absence of a test sample; and

comparing levels of activity of zsig28 in the presence and absence of a test sample, by a biological or biochemical assay; and

determining from the comparison, the presence of modulator of zsig28 activity in the test sample.

```
                10        20        30        40        50        60        70
zsig28 MSTTTCQVVAFLLSILGLAGCIAATGMDMWSTQDL-YDNPVTSVFQ--YEGLWRSCVRQSSGFTECRPYF : 67
CLAUD1 MANAGLQLLGFILASLGWIGSIVSTALPQWKIYSYAGDNIVTAQAI--YEGLWMSCVSQSTGQIQCKVFD : 68
AB0007 MASMGLQVLGISLAVLGWLGIILSCALPMWRVTAFIGSNIVTAQTS--WEGLWMNCVVQSTGQMQCKMYD : 68
HSU899 MASTASEIIAFMVSISGWVLVSSTLPTDYWKVSTIDGTVITTA-TY--WANLWKACVTDSTGVSNCKDFP : 67
AF0009 MGSAALEILGLVLCLVGWGGLILACGLPMWQVTAFLDHNIVTAQTT--WKGLWMSCVVQSTGHMQCKVYD : 68
AF0688 MVATCLQVVGFVTSFVGWIGVIVTTSTNDWVVTCGYTIPTCRKLDELGSKGLWADCVM-ATGLYHCKPLV : 69
AF0721 MASLGVQLVGYILGLLGLLGTSIAMLLPNWRTSSYVGASIVTAVGF--SKGLWMECATHSTGITQCDIYS : 68
PIR_A3 -MSMSLEITGTSLAVLGWLCTIVCCALPMWRVSAFIGSSIITAQIT--WEGLWMNCV-QSTGQMQCKMYD : 66

                80        90        100       110       120       130       140
zsig28 TILGLPAMLQAVRALMIVGIVLGAIGLLVSIFALKCIRIGSMEDSAKANMTLTSGIMFIVSGLCAIAGVS : 137
CLAUD1 SLLNLNSTLQATRALMVIGILLGLIAIFVSTIGMKCMRCLEDDEVQKMWMAVIGGIIFLISGLATLVATA : 138
AB0007 SMLALPQDLQAARALMVISIIVGALGMLLSVVGGKCTNCME-DETVKAKIMITAGAVFIVASMLIMVPVS : 137
HSU899 SMLALDGYIQACRGLMIAAVSLGFFGSIFALFGMKCTKVGG-SDKAKAKIACLAGIVFILSGLCSMTGCS : 136
AF0009 SVLALSTEVQAARALTVSAVLLAFVALFVTLAGAQCTTCVA-PGPAKARVALTGGVLYLFCGLLALVPLC : 137
AF0688 DILILPGYVQACRALMIAASVLGLPAILLLLTVLPCIRMGQEPGVAKYRRAQLAGVLLILLALCALVATI : 139
AF0721 TLLGLPADIQAAQAMMVTSSAMSSLACIISVVGMRCT-VFCQDSRAKDRVAVVGGVFFILGGILGFIPVA : 137
PIR_A3 SLLALPQDLQAARALIVVSILLAAFGLLVALVGAQCTNC-VQDETAKAKITIVAGVLFLLAAVLTLVPVS : 135
                    TMTMTMTMTMTMTMTMTMT                   TMTMTMTMTMTMTMT
```

FIG. 1A

```
            150       160       170       180       190       200       210
zsig28 VFANMLVTNFWMSTANMYTGMGGMVQTVQTRYTFGAALFVGWVAGGLTLIGGVMMCIACRGLAPEETNYK ; 207
CLAUD1 WYGNRIVQEFYDPLT-----------PINARYEFGQALFTGWAAASLCLLGGVLLSCSCPRKTTSYPTPR ; 197
AB0007 WTAHNVIRDFYNPMVA----------SGQKR-EMGASLYVGWAASGLLLLGGGLLCCSCPP-RSNDKPYS ; 195
HSU899 LYANKITTEFFDPLF-----------VEQKYELGAALFIGWAGASLCIIGGVIFCFSISDNNKTPRYTY ; 194
AF0009 WFANIVVREFYDPSVP----------VSQKYELGAALYIGWAATALLMVGGCLLCCGAWVCTGRPDLSF ; 196
AF0688 WFPVCAHRETT------------------IVSFGYSLYAGWIGAVLCLVGGCVILCCAGDAQAFGENVS ; 190
AF0721 WNLHGILRDFYSPLVP----------DSMKFEIGEALYLGIISALFSLVAGVILCFSCSPQGNRTNYYD ; 196
PIR_A3 WSANTIIRDFYNPLVP----------EAQKR-EMGTGLYVGWAAAALQLLGGALLCCSCPPREKYAPTKI ; 194
       TMT                                TMTMTMTMTMTMTMTMTM

            220       230       240       250       260       270       280
zsig28 AVSYHASGHSVAYKPGGFKASTGFGSNTKNKKIYDGGARTEDEVQSYPSKHDYV---------------- ; 261
CLAUD1 PYPKPTPSSGKDYV---------------------------------------------------------- ; 211
AB0007 AKYSAARSVPASNYV---------------------------------------------------------- ; 210
HSU899 NGATSVMSSRTKYHGGEDFKTTNPS------------------------------------------------ ; 219
AF0009 PVKYSAPRRPTATGDYDKKNYV---------------------------------------------------- ; 218
AF0688 TTLRALAPRLMRRVPTYKRAARLPTEVL------------------------------------------------ ; 218
AF0721 GYQAQPLATRSSPRSAQQPKAKSEFNSYSLTG------------------------------------------ ; 228
PIR_A3 LYSAPRSTGPGTGTGTAYDRKTTSERPGARTPHHHHYQPSMYPTRPACSLASETTPPSRRLQTPRSLLAR ; 264

            290
zsig28 ---------------- ; 261
CLAUD1 ---------------- ; 211
AB0007 ---------------- ; 210
HSU899 ---------------- ; 219
AF0009 ---------------- ; 218
AF0688 ---------------- ; 218
AF0721 ---------------- ; 228
PIR_A3 LEEDRQPGVPFSPVAT ; 280
```

FIG. 1B

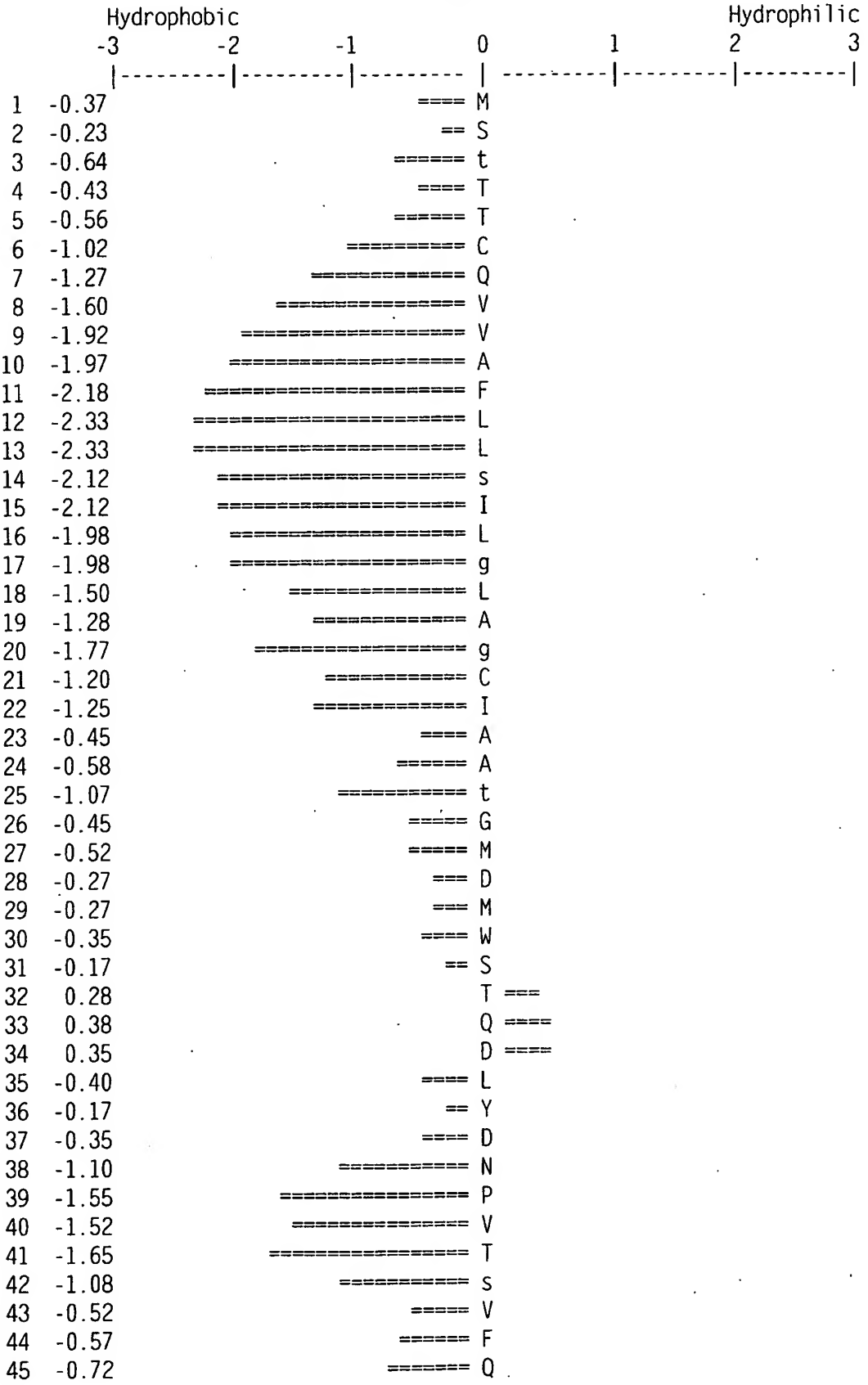

Hydrophobic
Hydrophilic
-3
-2
-1
0
1
2
3
1 -0.37 M
2 -0.23 S
3 -0.64 t
4 -0.43 T
5 -0.56 T
6 -1.02 C
7 -1.27 Q
8 -1.60 V
9 -1.92 V
10 -1.97 A
11 -2.18 F
12 -2.33 L
13 -2.33 L
14 -2.12 s
15 -2.12 I
16 -1.98 L
17 -1.98 g
18 -1.50 L
19 -1.28 A
20 -1.77 g
21 -1.20 C
22 -1.25 I
23 -0.45 A
24 -0.58 A
25 -1.07 t
26 -0.45 G
27 -0.52 M
28 -0.27 D
29 -0.27 M
30 -0.35 W
31 -0.17 S
32 0.28 T
33 0.38 Q
34 0.35 D
35 -0.40 L
36 -0.17 Y
37 -0.35 D
38 -1.10 N
39 -1.55 P
40 -1.52 V
41 -1.65 T
42 -1.08 s
43 -0.52 V
44 -0.57 F
45 -0.72 Q

FIG. 2A

46	-0.25	===	Y
47	0.18		E ==
48	-0.48	=====	G
49	-0.73	=======	L
50	0.07		W =
51	0.67		R =======
52	0.22		S ==
53	0.22		C ==
54	0.38		V ====
55	0.22		R ==
56	-0.35	====	Q
57	0.12		S =
58	-0.10	=	S
59	0.35		G ====
60	0.35		F ====
61	0.38		T ====
62	0.03		E
63	-0.53	=====	C
64	-0.67	=======	R
65	-1.47	==========	P
66	-1.47	==========	Y
67	-1.38	==========	F
68	-0.97	=========	T
69	-0.98	=========	I
70	-0.90	========	L
71	-0.90	========	G
72	-0.87	========	L
73	-0.65	=======	P
74	-0.90	========	A
75	-0.32	===	M
76	-0.18	==	L
77	-0.18	==	Q
78	-0.43	====	A
79	-0.65	=======	V
80	-0.65	=======	R
81	-1.72	==========	A
82	-1.93	==========	L
83	-1.88	==========	M
84	-1.97	==========	I
85	-2.23	==========	V
86	-2.07	==========	g
87	-1.80	==========	I
88	-2.07	==========	V
89	-2.12	==========	L
90	-2.12	==========	g
91	-1.80	==========	A
92	-2.28	==========	I
93	-2.28	==========	g

FIG. 2B

94	-2.13	==========	L
95	-1.92	==========	L
96	-1.92	==========	V
97	-1.17	==========	s
98	-0.77	==========	I
99	-0.77	==========	F
100	0.15	==	A
101	-0.07	=	L
102	0.23	==	K
103	-0.22	==	C
104	-0.27	===	I
105	0.53	=====	R
106	0.53	=====	I
107	0.88	=========	G
108	0.80	========	S
109	1.25	==========	M
110	1.38	==========	E
111	0.92	=========	D
112	0.20	==	S
113	0.08	=	A
114	-0.13	=	K
115	-0.70	==========	A
116	-1.18	==========	N
117	-1.22	==========	M
118	-1.30	==========	T
119	-1.45	==========	L
120	-1.57	==========	T
121	-1.80	==========	s
122	-1.48	==========	G
123	-2.05	==========	I
124	-1.75	==========	M
125	-1.83	==========	F
126	-1.58	==========	I
127	-1.37	==========	V
128	-1.42	==========	s
129	-0.93	=========	G
130	-1.50	==========	L
131	-1.45	==========	C
132	-1.23	==========	A
133	-1.40	==========	I
134	-1.52	==========	A
135	-1.52	==========	g
136	-0.92	=========	V
137	-0.88	=========	S
138	-1.23	==========	V
139	-1.23	==========	F
140	-0.88	=========	A
141	-0.77	=========	N

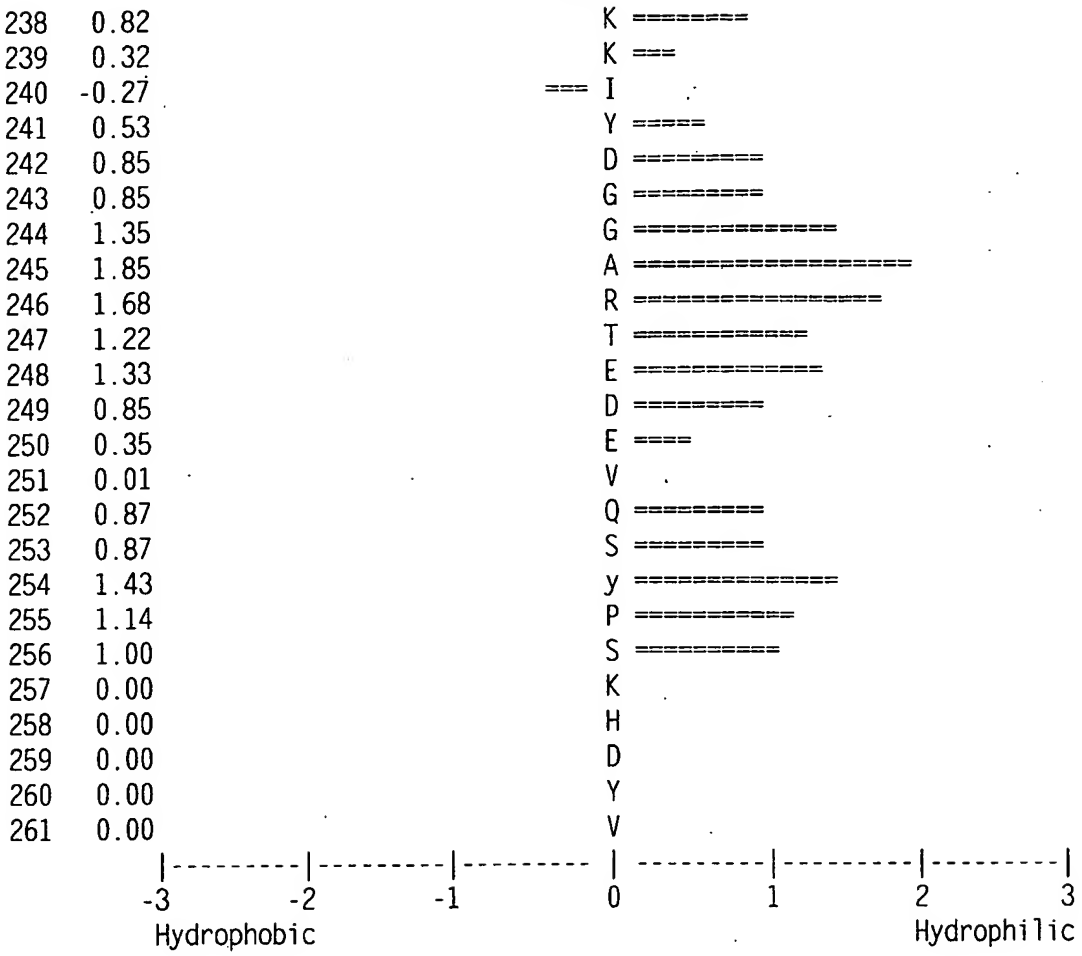
FIG. 2C

142	-1.22	========== M
143	-1.57	========== L
144	-1.48	========== V
145	-1.18	========== T
146	-1.18	========== N
147	-1.30	========== F
148	-0.85	========= W
149	-0.50	===== M
150	-0.67	======= S
151	-0.78	======= T
152	-0.72	======= A
153	-0.85	========= N
154	-1.45	========== M
155	-1.23	========== Y
156	-1.07	========== T
157	-1.25	========== G
158	-1.22	========== M
159	-1.07	========== g
160	-0.75	======= G
161	-0.72	======= M
162	-0.57	====== V
163	0.18	Q ==
164	-0.23	== T
165	-0.23	== V
166	-0.40	==== Q
167	-1.00	========== T
168	-1.02	========== R
169	-1.60	========== Y
170	-1.52	========== T
171	-1.87	========== F
172	-1.70	========== g
173	-1.13	========== A
174	-1.62	========== A
175	-1.78	========== L
176	-1.57	========== F
177	-1.72	========== V
178	-1.47	========== G
179	-1.77	========== W
180	-1.77	========== V
181	-1.82	========== A
182	-2.03	========== g
183	-2.03	========== G
184	-2.03	========== L
185	-1.98	========== t
186	-1.63	========== L
187	-1.55	========== I
188	-1.42	========== g
189	-1.15	========== G

FIG. 2D

190	-1.23	========== V
191	-1.15	========== M
192	-0.43	==== M
193	-0.22	== C
194	-0.35	==== I
195	-0.13	= A
196	-0.05	C
197	0.62	R ======
198	0.62	G ======
199	0.55	L ======
200	0.88	A =========
201	0.98	P =========
202	1.48	E ==========
203	0.90	E =========
204	0.15	T ==
205	0.27	N ===
206	-0.15	= y
207	-0.25	=== K
208	-0.83	======== A
209	-0.70	======= V
210	-0.45	===== S
211	-0.18	== Y
212	0.25	H ===
213	0.08	A =
214	0.08	S =
215	-0.35	==== G
216	0.15	h ==
217	-0.17	== S
218	-0.22	== V
219	0.03	A
220	-0.30	=== Y
221	0.58	K ======
222	0.00	P
223	0.05	G =
224	-0.52	===== G
225	-0.52	===== F
226	-0.52	===== K
227	-1.02	========== A
228	-0.88	========= S
229	-0.90	========= t
230	-0.40	==== G
231	0.10	F =
232	0.55	G ======
233	1.05	S ==========
234	1.50	N ==========
235	1.17	T ==========
236	0.85	K ========
237	0.85	N ========

FIG. 2E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261 0.00
K
K
I
Y
D
G
G
A
R
T
E
D
E
V
Q
S
y
P
S
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D
Y
V
-3
-2
-1
0
1
2
3
Hydrophobic
Hydrophilic

FIG. 2F

SEQUENCE LISTING

<110> ZymoGenetics, Inc.
1201 Eastlake Avenue East
Seattle, Washington 98102
United States of America

<120> Stomach polypeptide zsig28

<130> 98-47

<150> US 60/100,656
<151> 1998-09-16

<160> 16

<170> FastSEQ for Windows Version 3.0

<210> 1
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          Met Ser Thr Thr Thr Cys Gln Val Val Ala Phe Leu Leu Ser
           1               5                  10

atc ctg ggg ctg gcc ggc tgc atc gcg gcc acc ggg atg gac atg tgg      159
Ile Leu Gly Leu Ala Gly Cys Ile Ala Ala Thr Gly Met Asp Met Trp
 15                  20                  25                  30

agc acc cag gac ctg tac gac aac ccc gtc acc tcc gtg ttc cag tac      207
Ser Thr Gln Asp Leu Tyr Asp Asn Pro Val Thr Ser Val Phe Gln Tyr
                35                  40                  45

gaa ggg ctc tgg agg agc tgc gtg agg cag agt tca ggc ttc acc gaa      255
Glu Gly Leu Trp Arg Ser Cys Val Arg Gln Ser Ser Gly Phe Thr Glu
            50                  55                  60
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tgc agg ccc tat ttc acc atc ctg gga ctt cca gcc atg ctg cag gca      303
Cys Arg Pro Tyr Phe Thr Ile Leu Gly Leu Pro Ala Met Leu Gln Ala
        65                  70                  75

gtg cga gcc ctg atg atc gta ggc atc gtc ctg ggt gcc att ggc ctc      351
Val Arg Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu
     80                  85                  90

ctg gta tcc atc ttt gcc ctg aaa tgc atc cgc att ggc agc atg gag      399
Leu Val Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu
 95                 100                 105                 110

gac tct gcc aaa gcc aac atg aca ctg acc tcc ggg atc atg ttc att      447
Asp Ser Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile
                115                 120                 125

gtc tca ggt ctt tgt gca att gct gga gtg tct gtg ttt gcc aac atg      495
Val Ser Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met
            130                 135                 140

ctg gtg act aac ttc tgg atg tcc aca gct aac atg tac acc ggc atg      543
Leu Val Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met
        145                 150                 155

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Gly Gly Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala
    160                 165                 170

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Leu Phe Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val
175                 180                 185                 190

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Met Met Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr
                195                 200                 205

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Lys Ala Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro
            210                 215                 220

gga ggc ttc aag gcc agc act ggc ttt ggg tcc aac acc aaa aac aag      783
Gly Gly Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys
        225                 230                 235

aag ata tac gat gga ggt gcc cgc aca gag gac gag gta caa tct tat      831
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Pro Ser Lys His Asp Tyr Val
255                 260

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Gln Asp Leu Tyr Asp Asn Pro Val Thr Ser Val Phe Gln Tyr Glu Gly
        35                  40                  45
Leu Trp Arg Ser Cys Val Arg Gln Ser Ser Gly Phe Thr Glu Cys Arg
    50                  55                  60
Pro Tyr Phe Thr Ile Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65                  70                  75                  80
Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
                85                  90                  95
Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
            100                 105                 110
Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
        115                 120                 125
Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val
    130                 135                 140
Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145                 150                 155                 160
Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
                165                 170                 175
Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
            180                 185                 190
Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
        195                 200                 205
Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
    210                 215                 220
```

```
Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225                 230                 235                 240
Tyr Asp Gly Gly Ala Arg Thr Glu Asp Glu Val Gln Ser Tyr Pro Ser
                245                 250                 255
Lys His Asp Tyr Val
            260
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<210> 3
<211> 211
<212> PRT
<213> Mus musculus

<400> 3

```
Met Ala Asn Ala Gly Leu Gln Leu Leu Gly Phe Ile Leu Ala Ser Leu
 1               5                  10                  15
Gly Trp Ile Gly Ser Ile Val Ser Thr Ala Leu Pro Gln Trp Lys Ile
            20                  25                  30
Tyr Ser Tyr Ala Gly Asp Asn Ile Val Thr Ala Gln Ala Ile Tyr Glu
        35                  40                  45
Gly Leu Trp Met Ser Cys Val Ser Gln Ser Thr Gly Gln Ile Gln Cys
    50                  55                  60
Lys Val Phe Asp Ser Leu Leu Asn Leu Asn Ser Thr Leu Gln Ala Thr
65                  70                  75                  80
Arg Ala Leu Met Val Ile Gly Ile Leu Leu Gly Leu Ile Ala Ile Phe
                85                  90                  95
Val Ser Thr Ile Gly Met Lys Cys Met Arg Cys Leu Glu Asp Asp Glu
            100                 105                 110
Val Gln Lys Met Trp Met Ala Val Ile Gly Gly Ile Ile Phe Leu Ile
        115                 120                 125
Ser Gly Leu Ala Thr Leu Val Ala Thr Ala Trp Tyr Gly Asn Arg Ile
    130                 135                 140
Val Gln Glu Phe Tyr Asp Pro Leu Thr Pro Ile Asn Ala Arg Tyr Glu
145                 150                 155                 160
Phe Gly Gln Ala Leu Phe Thr Gly Trp Ala Ala Ala Ser Leu Cys Leu
                165                 170                 175
Leu Gly Gly Val Leu Leu Ser Cys Ser Cys Pro Arg Lys Thr Thr Ser
            180                 185                 190
Tyr Pro Thr Pro Arg Pro Tyr Pro Lys Pro Thr Pro Ser Ser Gly Lys
        195                 200                 205
Asp Tyr Val
    210
```

<210> 4
<211> 210

<212> PRT
<213> Mus musculus

<400> 4
Met Ala Ser Met Gly Leu Gln Val Leu Gly Ile Ser Leu Ala Val Leu
1 5 10 15
Gly Trp Leu Gly Ile Ile Leu Ser Cys Ala Leu Pro Met Trp Arg Val
20 25 30
Thr Ala Phe Ile Gly Ser Asn Ile Val Thr Ala Gln Thr Ser Trp Glu
35 40 45
Gly Leu Trp Met Asn Cys Val Val Gln Ser Thr Gly Gln Met Gln Cys
50 55 60
Lys Met Tyr Asp Ser Met Leu Ala Leu Pro Gln Asp Leu Gln Ala Ala
65 70 75 80
Arg Ala Leu Met Val Ile Ser Ile Ile Val Gly Ala Leu Gly Met Leu
85 90 95
Leu Ser Val Val Gly Gly Lys Cys Thr Asn Cys Met Glu Asp Glu Thr
100 105 110
Val Lys Ala Lys Ile Met Ile Thr Ala Gly Ala Val Phe Ile Val Ala
115 120 125
Ser Met Leu Ile Met Val Pro Val Ser Trp Thr Ala His Asn Val Ile
130 135 140
Arg Asp Phe Tyr Asn Pro Met Val Ala Ser Gly Gln Lys Arg Glu Met
145 150 155 160
Gly Ala Ser Leu Tyr Val Gly Trp Ala Ala Ser Gly Leu Leu Leu Leu
165 170 175
Gly Gly Gly Leu Leu Cys Cys Ser Cys Pro Pro Arg Ser Asn Asp Lys
180 185 190
Pro Tyr Ser Ala Lys Tyr Ser Ala Ala Arg Ser Val Pro Ala Ser Asn
195 200 205
Tyr Val
210

<210> 5
<211> 219
<212> PRT
<213> Homo sapiens

<400> 5
Met Ala Ser Thr Ala Ser Glu Ile Ile Ala Phe Met Val Ser Ile Ser
1 5 10 15
Gly Trp Val Leu Val Ser Ser Thr Leu Pro Thr Asp Tyr Trp Lys Val
20 25 30
Ser Thr Ile Asp Gly Thr Val Ile Thr Thr Ala Thr Tyr Trp Ala Asn
35 40 45

```
Leu Trp Lys Ala Cys Val Thr Asp Ser Thr Gly Val Ser Asn Cys Lys
    50                  55                  60
Asp Phe Pro Ser Met Leu Ala Leu Asp Gly Tyr Ile Gln Ala Cys Arg
65                  70                  75                  80
Gly Leu Met Ile Ala Ala Val Ser Leu Gly Phe Phe Gly Ser Ile Phe
                85                  90                  95
Ala Leu Phe Gly Met Lys Cys Thr Lys Val Gly Gly Ser Asp Lys Ala
            100                 105                 110
Lys Ala Lys Ile Ala Cys Leu Ala Gly Ile Val Phe Ile Leu Ser Gly
        115                 120                 125
Leu Cys Ser Met Thr Gly Cys Ser Leu Tyr Ala Asn Lys Ile Thr Thr
    130                 135                 140
Glu Phe Phe Asp Pro Leu Phe Val Glu Gln Lys Tyr Glu Leu Gly Ala
145                 150                 155                 160
Ala Leu Phe Ile Gly Trp Ala Gly Ala Ser Leu Cys Ile Ile Gly Gly
                165                 170                 175
Val Ile Phe Cys Phe Ser Ile Ser Asp Asn Asn Lys Thr Pro Arg Tyr
            180                 185                 190
Thr Tyr Asn Gly Ala Thr Ser Val Met Ser Ser Arg Thr Lys Tyr His
        195                 200                 205
Gly Gly Glu Asp Phe Lys Thr Thr Asn Pro Ser
    210                 215
```

<210> 6
<211> 218
<212> PRT
<213> Homo sapiens

<400> 6

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Met Gly Ser Ala Ala Leu Glu Ile Leu Gly Leu Val Leu Cys Leu Val
 1               5                  10                  15
Gly Trp Gly Gly Leu Ile Leu Ala Cys Gly Leu Pro Met Trp Gln Val
            20                  25                  30
Thr Ala Phe Leu Asp His Asn Ile Val Thr Ala Gln Thr Thr Trp Lys
        35                  40                  45
Gly Leu Trp Met Ser Cys Val Val Gln Ser Thr Gly His Met Gln Cys
    50                  55                  60
Lys Val Tyr Asp Ser Val Leu Ala Leu Ser Thr Glu Val Gln Ala Ala
65                  70                  75                  80
Arg Ala Leu Thr Val Ser Ala Val Leu Leu Ala Phe Val Ala Leu Phe
                85                  90                  95
Val Thr Leu Ala Gly Ala Gln Cys Thr Thr Cys Val Ala Pro Gly Pro
            100                 105                 110
Ala Lys Ala Arg Val Ala Leu Thr Gly Gly Val Leu Tyr Leu Phe Cys
```

```
       115                 120                 125
Gly Leu Leu Ala Leu Val Pro Leu Cys Trp Phe Ala Asn Ile Val Val
    130                 135                 140
Arg Glu Phe Tyr Asp Pro Ser Val Pro Val Ser Gln Lys Tyr Glu Leu
145                 150                 155                 160
Gly Ala Ala Leu Tyr Ile Gly Trp Ala Ala Thr Ala Leu Leu Met Val
                165                 170                 175
Gly Gly Cys Leu Leu Cys Cys Gly Ala Trp Val Cys Thr Gly Arg Pro
            180                 185                 190
Asp Leu Ser Phe Pro Val Lys Tyr Ser Ala Pro Arg Arg Pro Thr Ala
        195                 200                 205
Thr Gly Asp Tyr Asp Lys Lys Asn Tyr Val
    210                 215
```

<210> 7
<211> 218
<212> PRT
<213> Homo sapiens

<400> 7

```
Met Val Ala Thr Cys Leu Gln Val Val Gly Phe Val Thr Ser Phe Val
 1               5                  10                  15
Gly Trp Ile Gly Val Ile Val Thr Thr Ser Thr Asn Asp Trp Val Val
            20                  25                  30
Thr Cys Gly Tyr Thr Ile Pro Thr Cys Arg Lys Leu Asp Glu Leu Gly
        35                  40                  45
Ser Lys Gly Leu Trp Ala Asp Cys Val Met Ala Thr Gly Leu Tyr His
    50                  55                  60
Cys Lys Pro Leu Val Asp Ile Leu Ile Leu Pro Gly Tyr Val Gln Ala
65                  70                  75                  80
Cys Arg Ala Leu Met Ile Ala Ala Ser Val Leu Gly Leu Pro Ala Ile
                85                  90                  95
Leu Leu Leu Leu Thr Val Leu Pro Cys Ile Arg Met Gly Gln Glu Pro
            100                 105                 110
Gly Val Ala Lys Tyr Arg Arg Ala Gln Leu Ala Gly Val Leu Leu Ile
        115                 120                 125
Leu Leu Ala Leu Cys Ala Leu Val Ala Thr Ile Trp Phe Pro Val Cys
    130                 135                 140
Ala His Arg Glu Thr Thr Ile Val Ser Phe Gly Tyr Ser Leu Tyr Ala
145                 150                 155                 160
Gly Trp Ile Gly Ala Val Leu Cys Leu Val Gly Gly Cys Val Ile Leu
                165                 170                 175
Cys Cys Ala Gly Asp Ala Gln Ala Phe Gly Glu Asn Val Ser Thr Thr
            180                 185                 190
Leu Arg Ala Leu Ala Pro Arg Leu Met Arg Arg Val Pro Thr Tyr Lys
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195 200 205
Arg Ala Ala Arg Leu Pro Thr Glu Val Leu
210 215

<210> 8
<211> 228
<212> PRT
<213> Mus musculus

<400> 8
Met Ala Ser Leu Gly Val Gln Leu Val Gly Tyr Ile Leu Gly Leu Leu
1 5 10 15
Gly Leu Leu Gly Thr Ser Ile Ala Met Leu Leu Pro Asn Trp Arg Thr
20 25 30
Ser Ser Tyr Val Gly Ala Ser Ile Val Thr Ala Val Gly Phe Ser Lys
35 40 45
Gly Leu Trp Met Glu Cys Ala Thr His Ser Thr Gly Ile Thr Gln Cys
50 55 60
Asp Ile Tyr Ser Thr Leu Leu Gly Leu Pro Ala Asp Ile Gln Ala Ala
65 70 75 80
Gln Ala Met Met Val Thr Ser Ser Ala Met Ser Ser Leu Ala Cys Ile
85 90 95
Ile Ser Val Val Gly Met Arg Cys Thr Val Phe Cys Gln Asp Ser Arg
100 105 110
Ala Lys Asp Arg Val Ala Val Val Gly Gly Val Phe Phe Ile Leu Gly
115 120 125
Gly Ile Leu Gly Phe Ile Pro Val Ala Trp Asn Leu His Gly Ile Leu
130 135 140
Arg Asp Phe Tyr Ser Pro Leu Val Pro Asp Ser Met Lys Phe Glu Ile
145 150 155 160
Gly Glu Ala Leu Tyr Leu Gly Ile Ile Ser Ala Leu Phe Ser Leu Val
165 170 175
Ala Gly Val Ile Leu Cys Phe Ser Cys Ser Pro Gln Gly Asn Arg Thr
180 185 190
Asn Tyr Tyr Asp Gly Tyr Gln Ala Gln Pro Leu Ala Thr Arg Ser Ser
195 200 205
Pro Arg Ser Ala Gln Gln Pro Lys Ala Lys Ser Glu Phe Asn Ser Tyr
210 215 220
Ser Leu Thr Gly
225

<210> 9
<211> 280
<212> PRT
<213> Ratus norvegicus

<400> 9

```
Met Ser Met Ser Leu Glu Ile Thr Gly Thr Ser Leu Ala Val Leu Gly
 1               5                  10                  15
Trp Leu Cys Thr Ile Val Cys Cys Ala Leu Pro Met Trp Arg Val Ser
            20                  25                  30
Ala Phe Ile Gly Ser Ser Ile Ile Thr Ala Gln Ile Thr Trp Glu Gly
        35                  40                  45
Leu Trp Met Asn Cys Val Gln Ser Thr Gly Gln Met Gln Cys Lys Met
    50                  55                  60
Tyr Asp Ser Leu Leu Ala Leu Pro Gln Asp Leu Gln Ala Ala Arg Ala
65                  70                  75                  80
Leu Ile Val Val Ser Ile Leu Leu Ala Ala Phe Gly Leu Leu Val Ala
                85                  90                  95
Leu Val Gly Ala Gln Cys Thr Asn Cys Val Gln Asp Glu Thr Ala Lys
            100                 105                 110
Ala Lys Ile Thr Ile Val Ala Gly Val Leu Phe Leu Leu Ala Ala Val
        115                 120                 125
Leu Thr Leu Val Pro Val Ser Trp Ser Ala Asn Thr Ile Ile Arg Asp
    130                 135                 140
Phe Tyr Asn Pro Leu Val Pro Glu Ala Gln Lys Arg Glu Met Gly Thr
145                 150                 155                 160
Gly Leu Tyr Val Gly Trp Ala Ala Ala Ala Leu Gln Leu Leu Gly Gly
                165                 170                 175
Ala Leu Leu Cys Cys Ser Cys Pro Pro Arg Glu Lys Tyr Ala Pro Thr
            180                 185                 190
Lys Ile Leu Tyr Ser Ala Pro Arg Ser Thr Gly Pro Gly Thr Gly Thr
        195                 200                 205
Gly Thr Ala Tyr Asp Arg Lys Thr Thr Ser Glu Arg Pro Gly Ala Arg
    210                 215                 220
Thr Pro His His His His Tyr Gln Pro Ser Met Tyr Pro Thr Arg Pro
225                 230                 235                 240
Ala Cys Ser Leu Ala Ser Glu Thr Thr Pro Pro Ser Arg Arg Leu Gln
                245                 250                 255
Thr Pro Arg Ser Leu Leu Ala Arg Leu Glu Glu Asp Arg Gln Pro Gly
            260                 265                 270
Val Pro Phe Ser Pro Val Ala Thr
        275                 280
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<210> 10
<211> 783
<212> DNA
<213> Artificial Sequence

<220>
<223> Degenerate nucleotide sequence of zsig28 polypeptide

<221> misc_feature
<222> (1)...(783)
<223> n = A,T,C or G

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tgyathgcng cnacnggnat ggayatgtgg wsnacncarg ayytntayga yaayccngtn 120
acnwsngtnt tycartayga rggnytntgg mgnwsntgyg tnmgncarws nwsnggntty 180
acngartgym gnccntaytt yacnathytn ggnytnccng cnatgytnca rgcngtnmgn 240
gcnytnatga thgtnggnat hgtnytnggn gcnathggny tnytngtnws naththygcn 300
ytnaartgya thmgnathgg nwsnatggar gaywsngcna argcnaayat gacnytnacn 360
wsnggnatha tgttyathgt nwsnggnytn tgygcnathg cnggngtnws ngtnttygcn 420
aayatgytng tnacnaaytt ytggatgwsn acngcnaaya tgtayacngg natgggnggn 480
atggtncara cngtncarac nmgntayacn ttyggngcng cnytnttygt nggntgggtn 540
gcnggnggny tnacnytnat hggnggngtn atgatgtgya thgcntgymg nggnytngcn 600
ccngargara cnaaytayaa rgcngtnwsn taycaygcnw snggncayws ngtngcntay 660
aarccnggng gnttyaargc nwsnacnggn ttyggnwsna ayacnaaraa yaaraarath 720
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gtn 783

<210> 11
<211> 17
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC447

<400> 11
taacaatttc acacagg 17

<210> 12
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC12501

<400> 12

gtatccatct ttgccctgaa 20

<210> 13
<211> 20
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC12502

<400> 13
gctaggatta caggcgtgag 20

<210> 14
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC976

<400> 14
cgttgtaaaa cgacggcc 18

<210> 15
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC19410

<400> 15
gcgggcggcc agtatcat 18

<210> 16
<211> 18
<212> DNA
<213> Artificial Sequence

<220>
<223> Oligonucleotide primer ZC19411

<400> 16
cggaggtgac ggggttgt 18